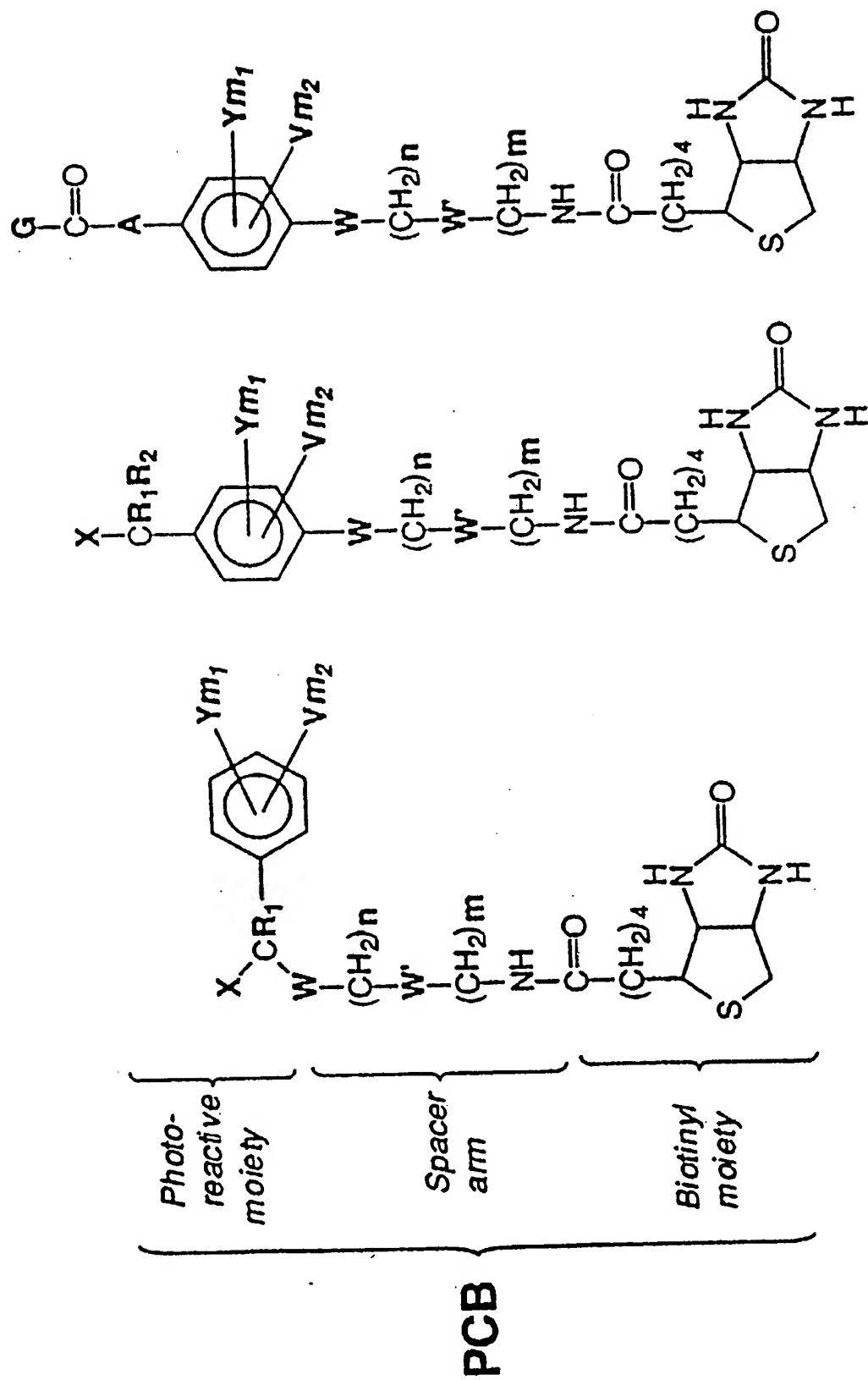


Figure 1



**Figure 2**

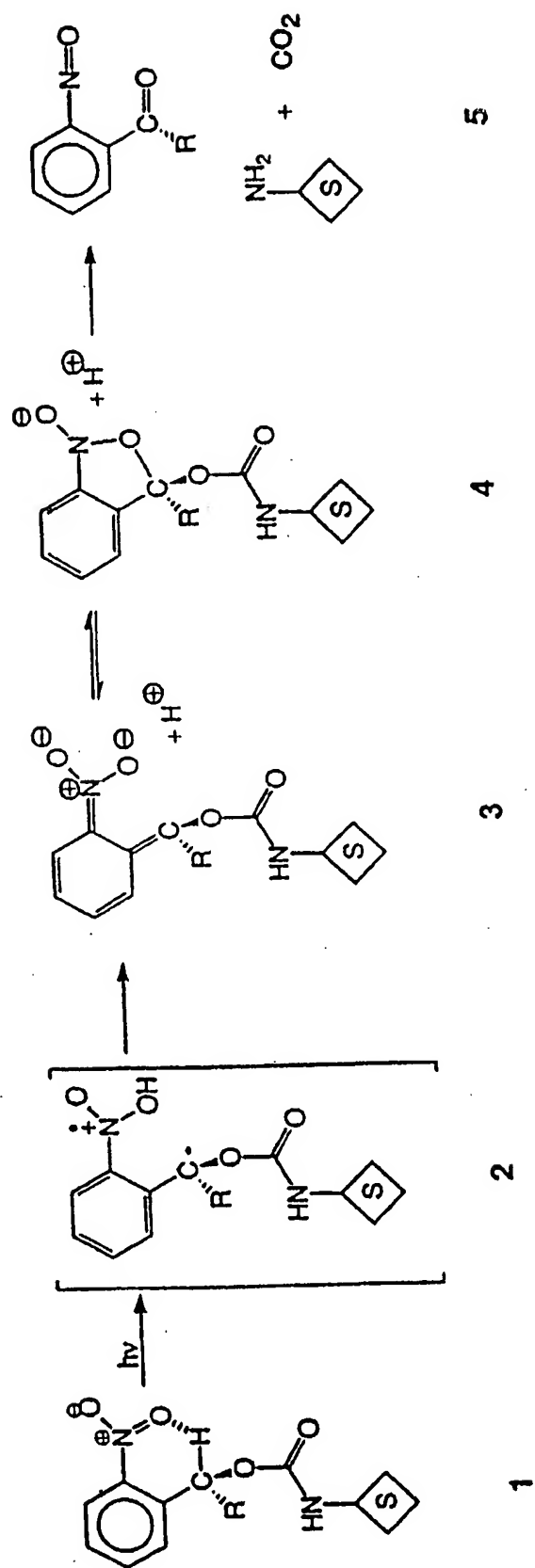


Figure 3

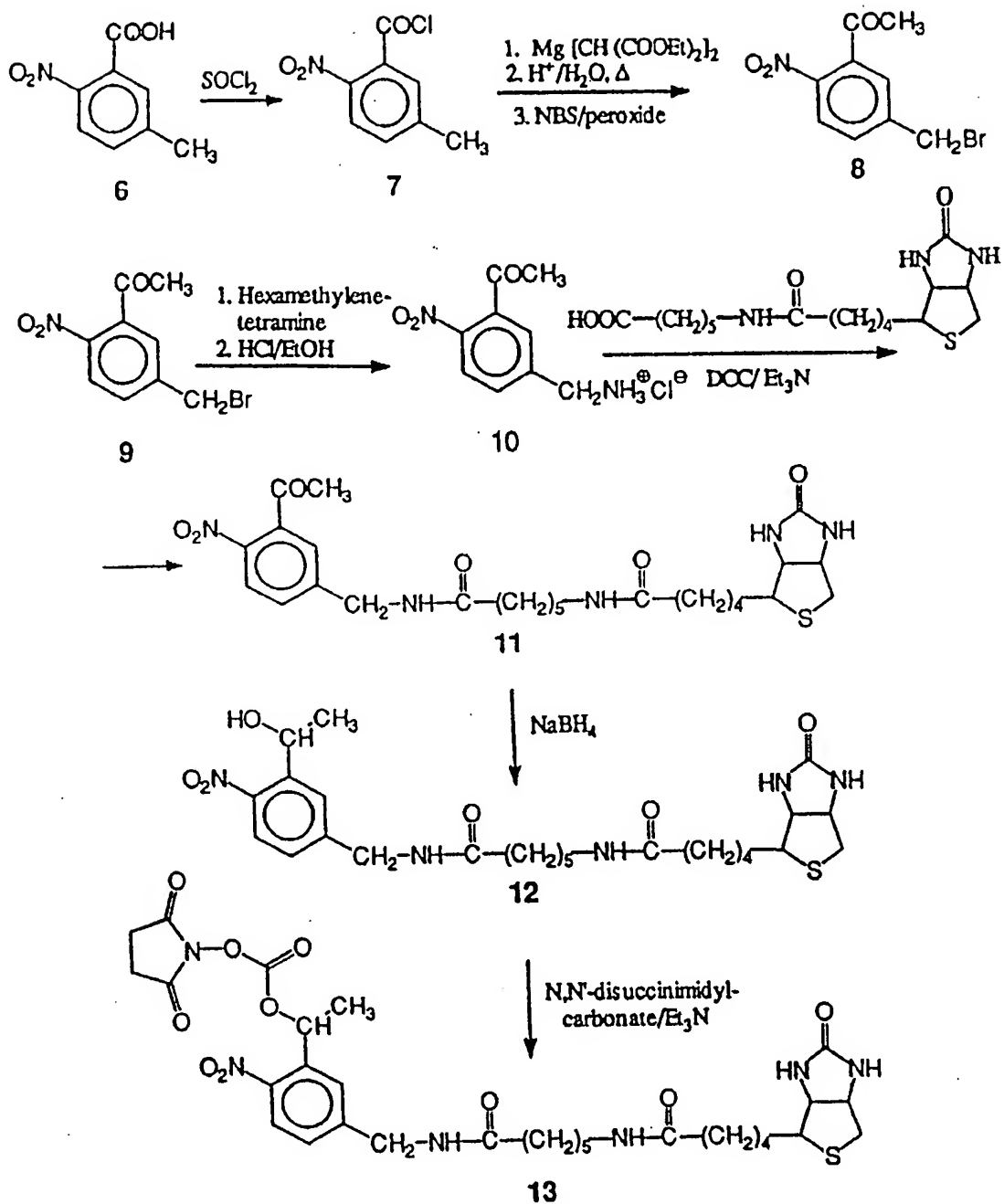
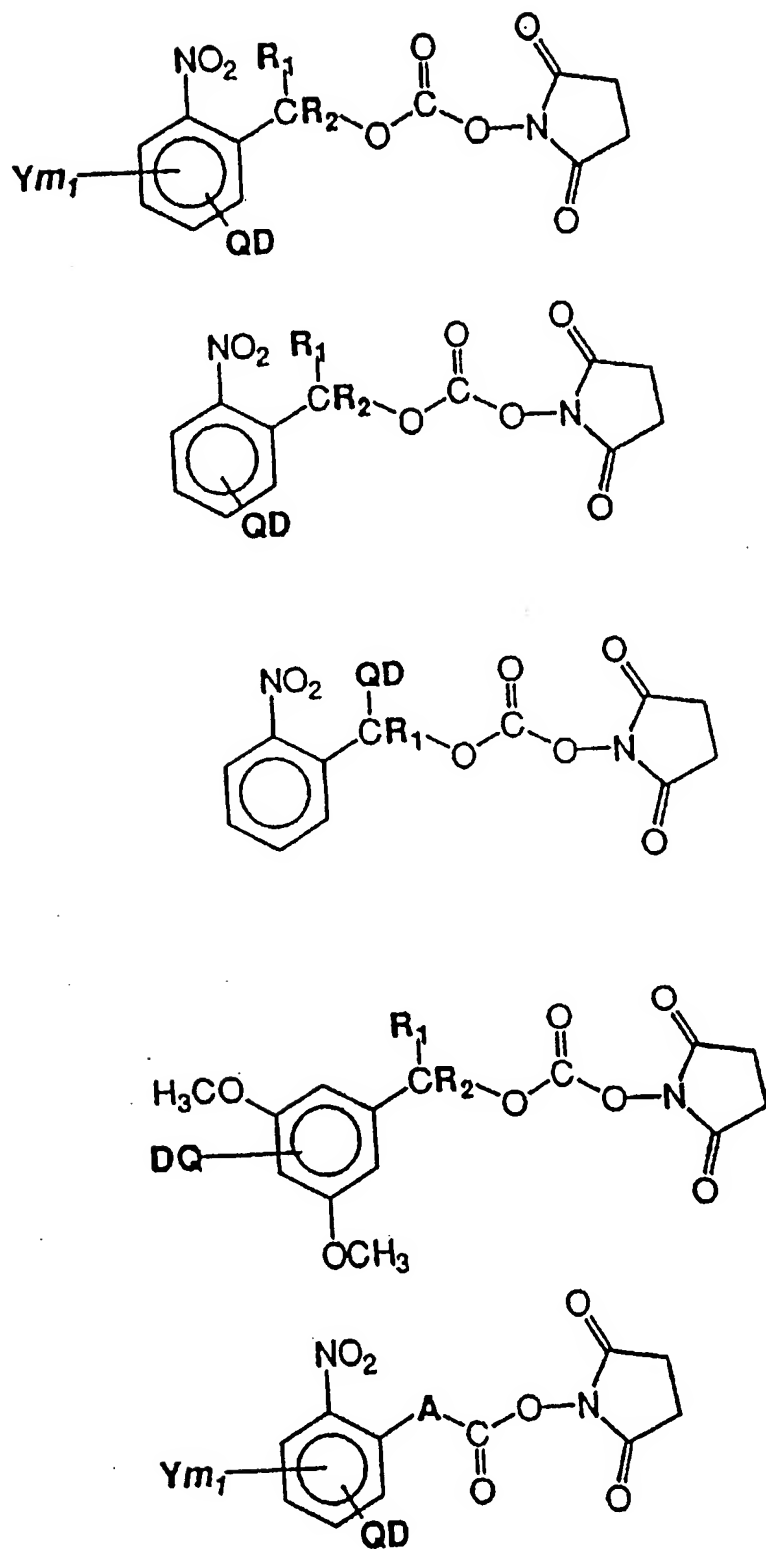
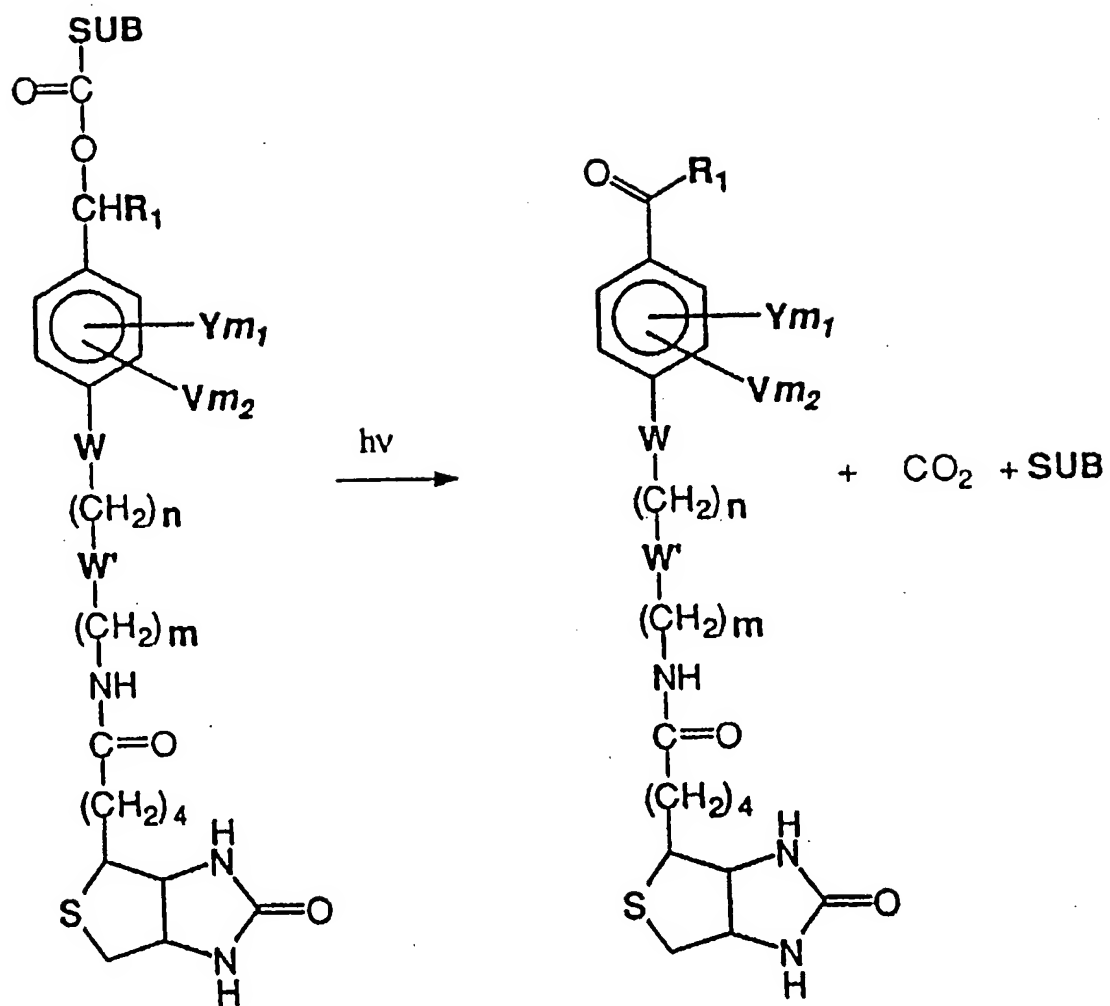


Figure 4



**Figure 5**



**Figure 6**

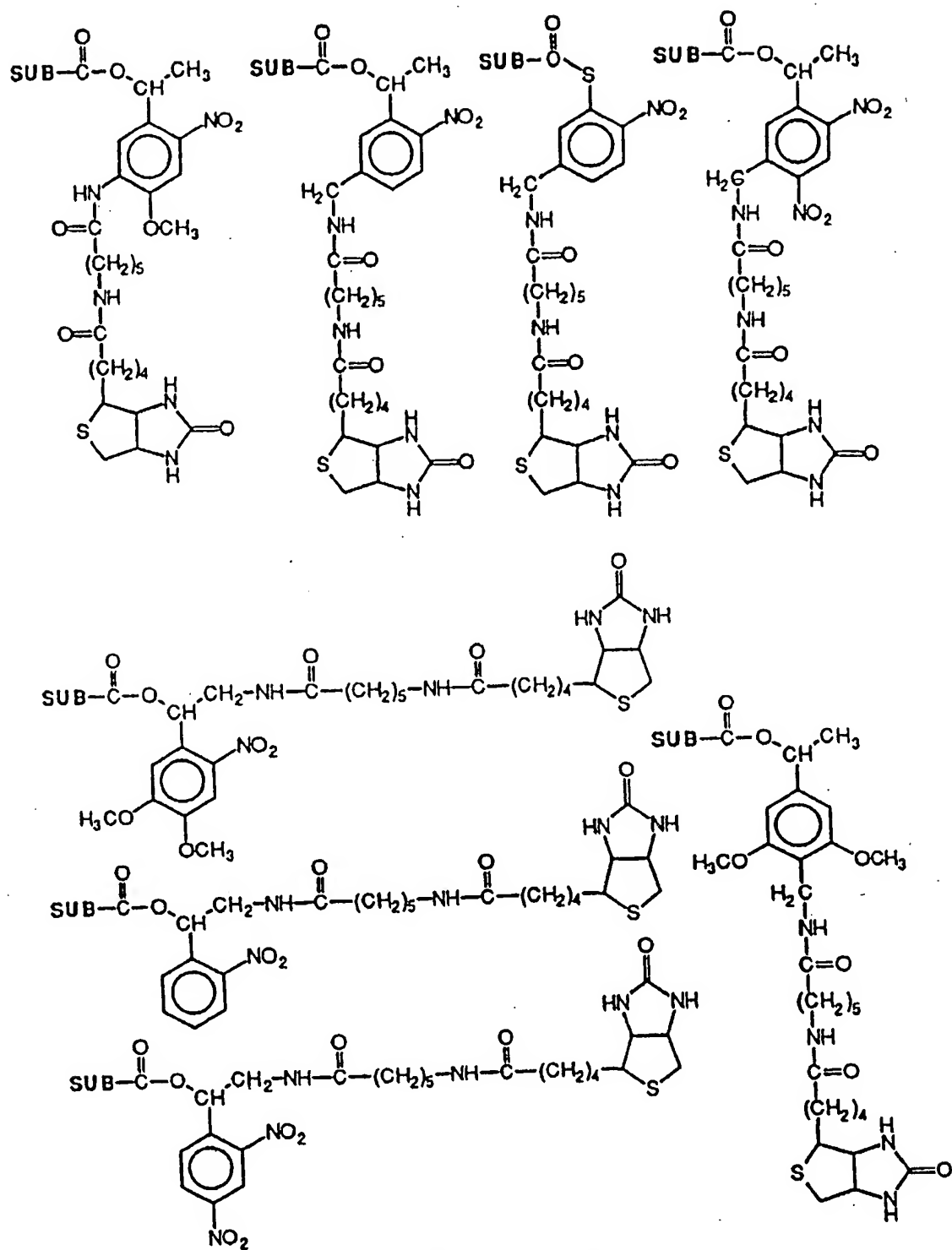


Figure 7

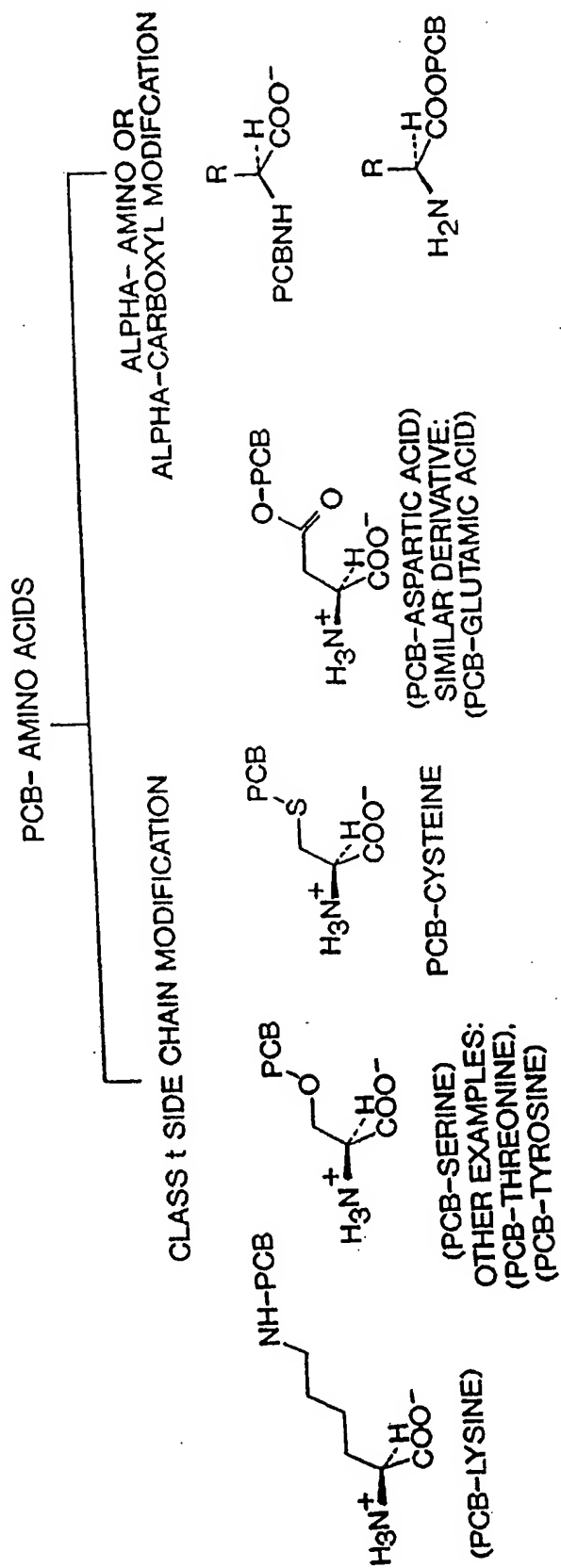
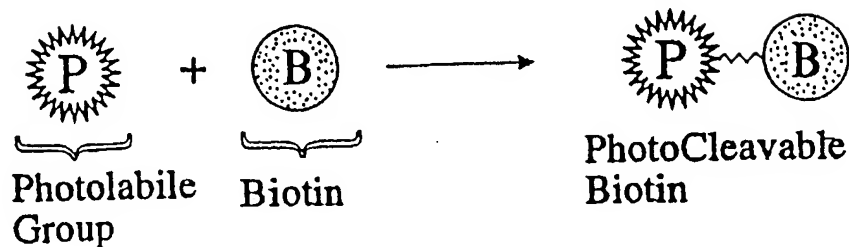


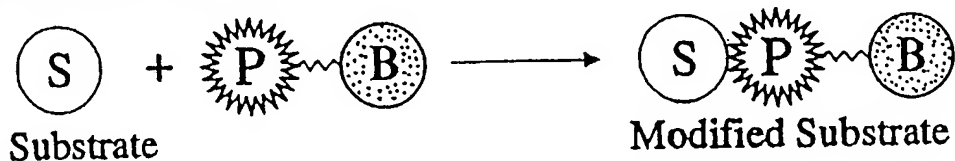
Figure 8



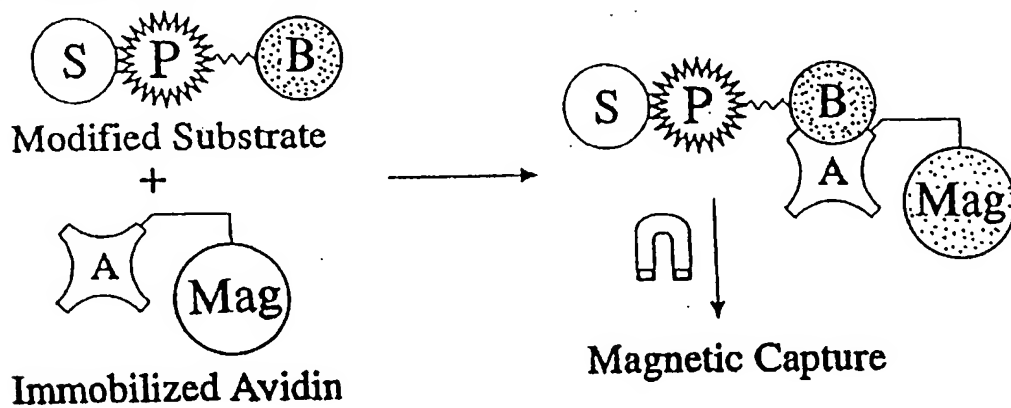
### Step 1: Synthesis of Photocleavable Biotin



### Step 2: Modification of the substrate by Photocleavable Biotin



### Step 3: Isolation of the Modified Substrate using Avidin



### Step 4: Detachment of Pure Substrate

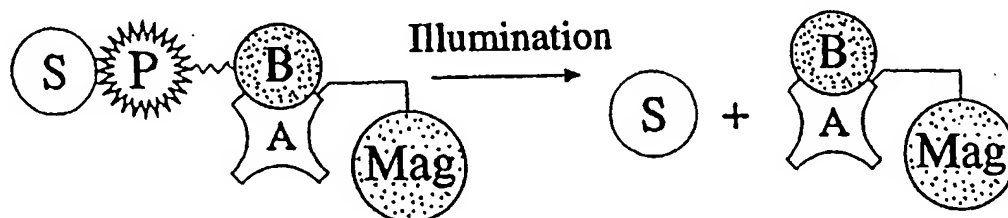
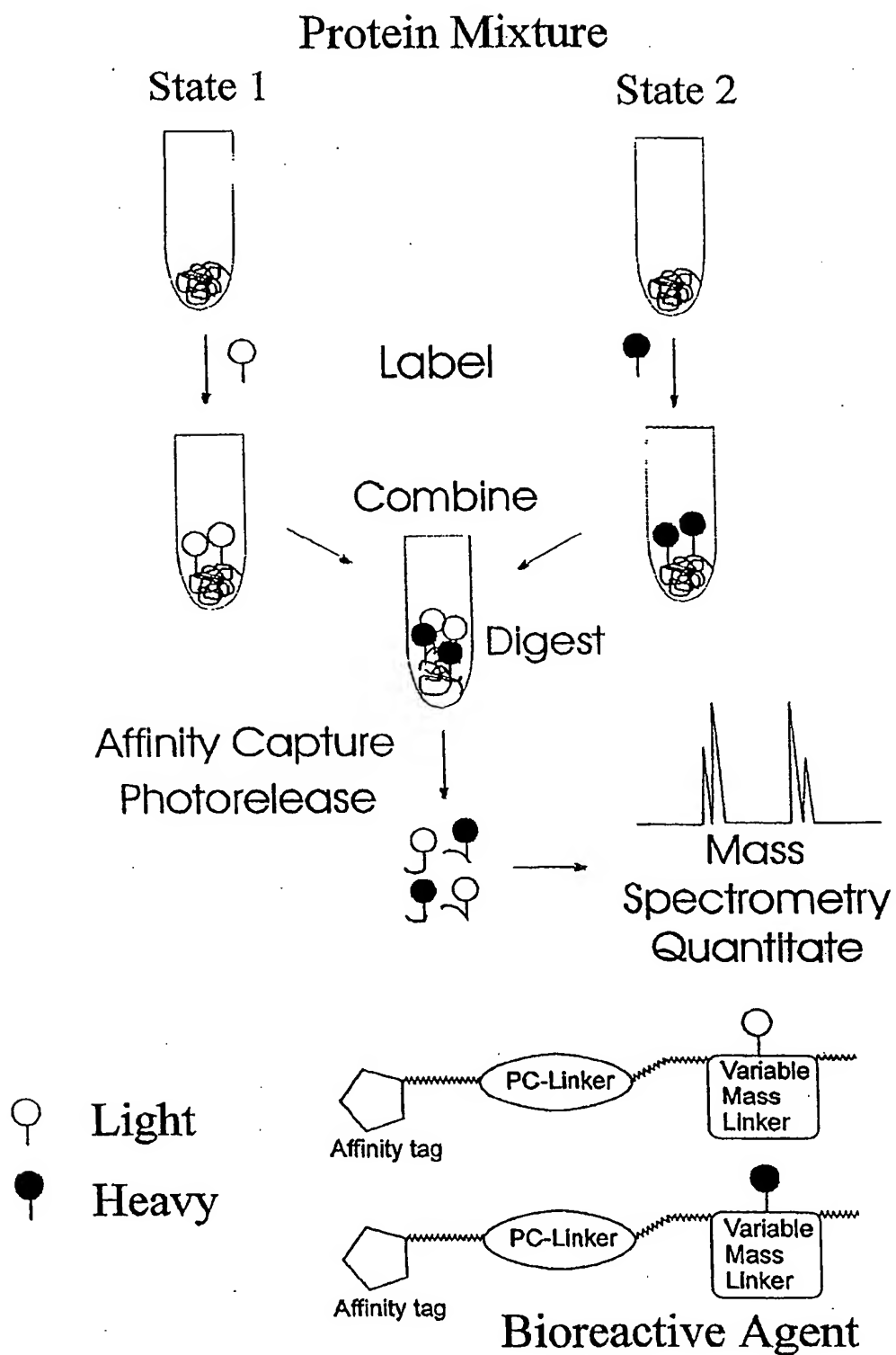
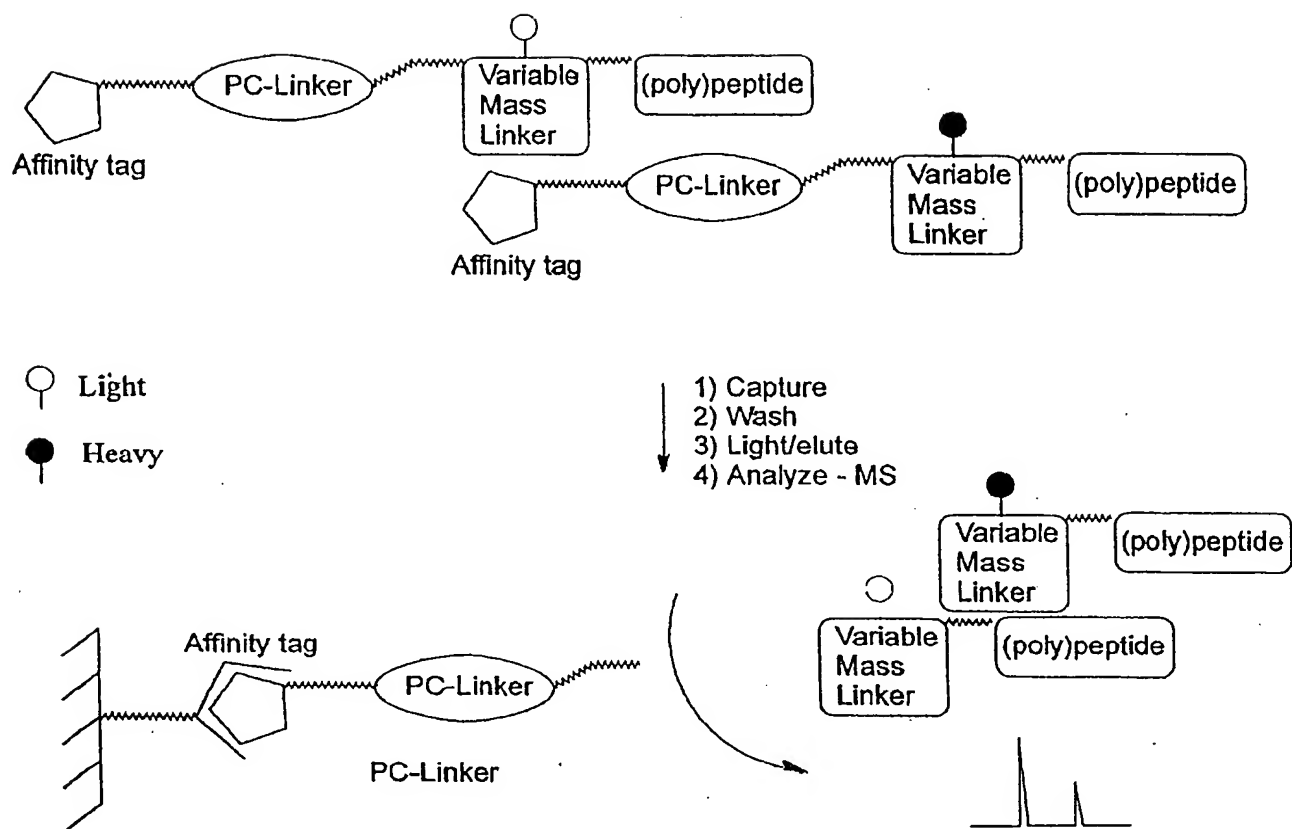


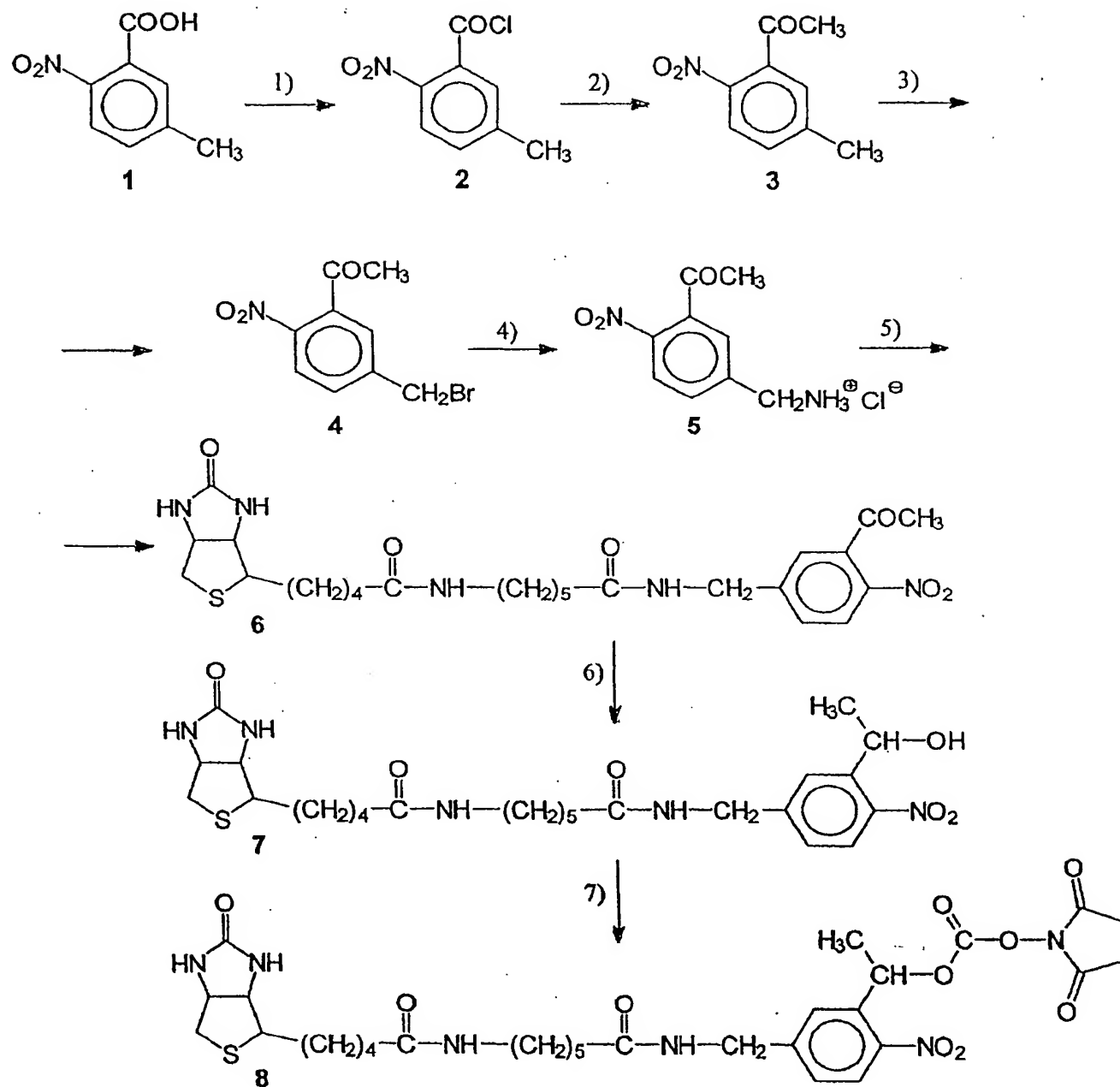
Figure 9



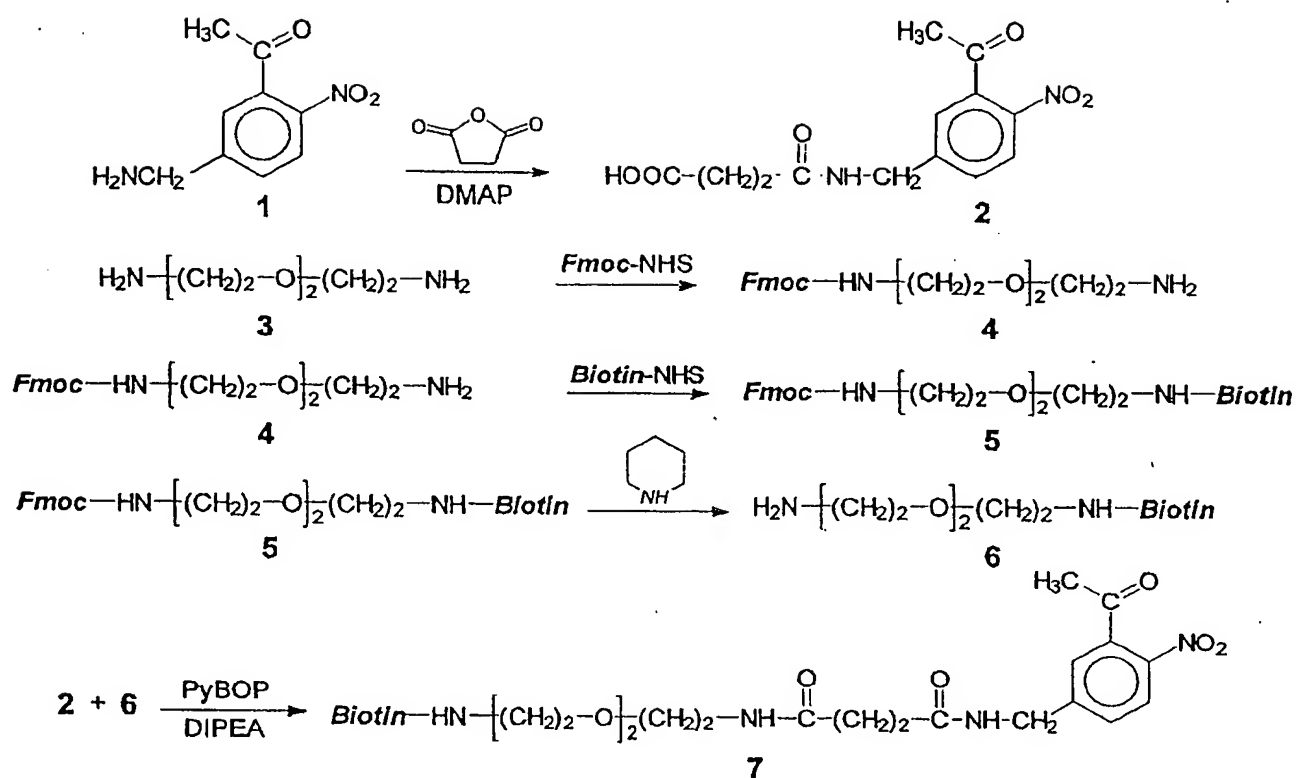
**Figure 10**



**Figure 11**

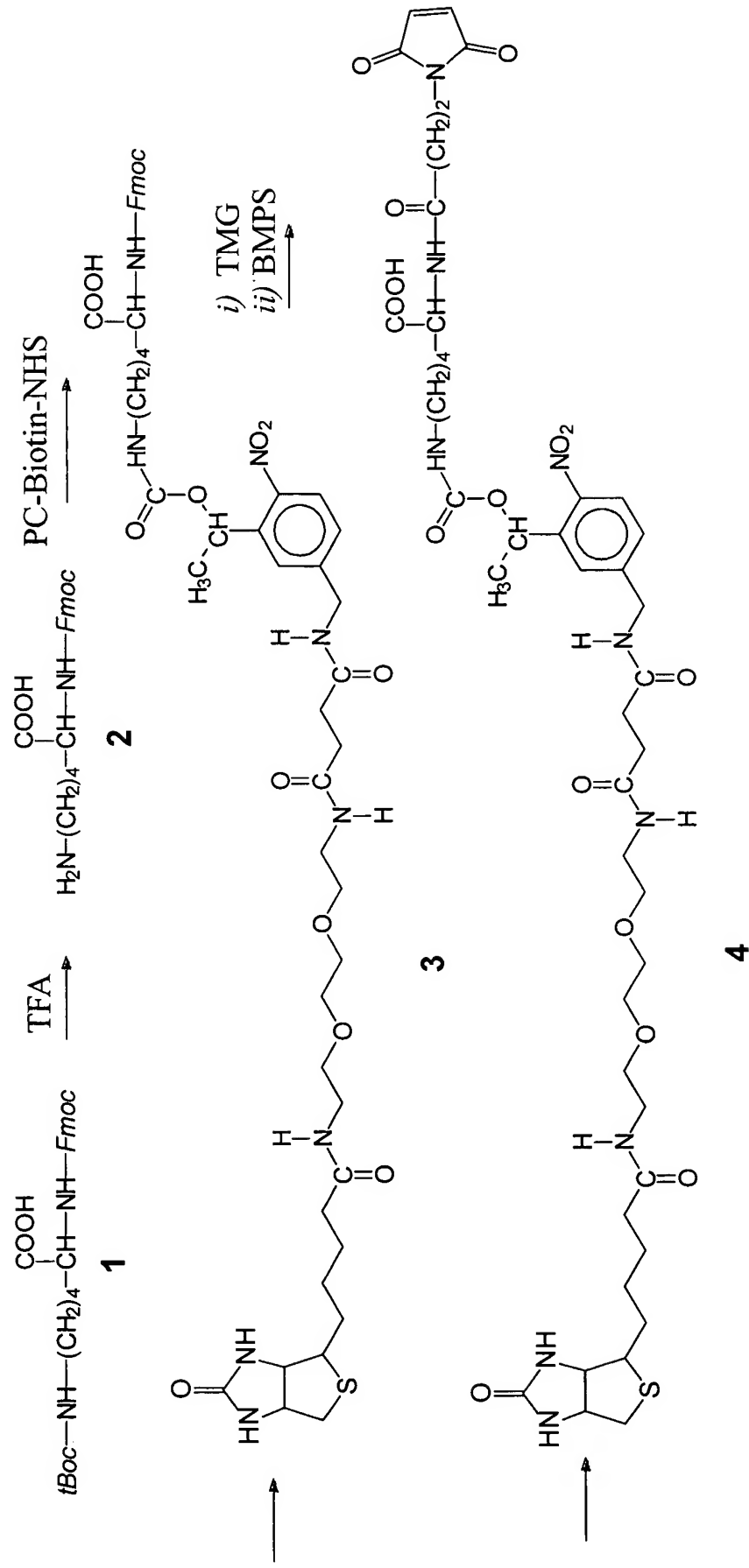


**Figure 12**



**Figure 13**

**Figure 14A**



**Figure 14B**

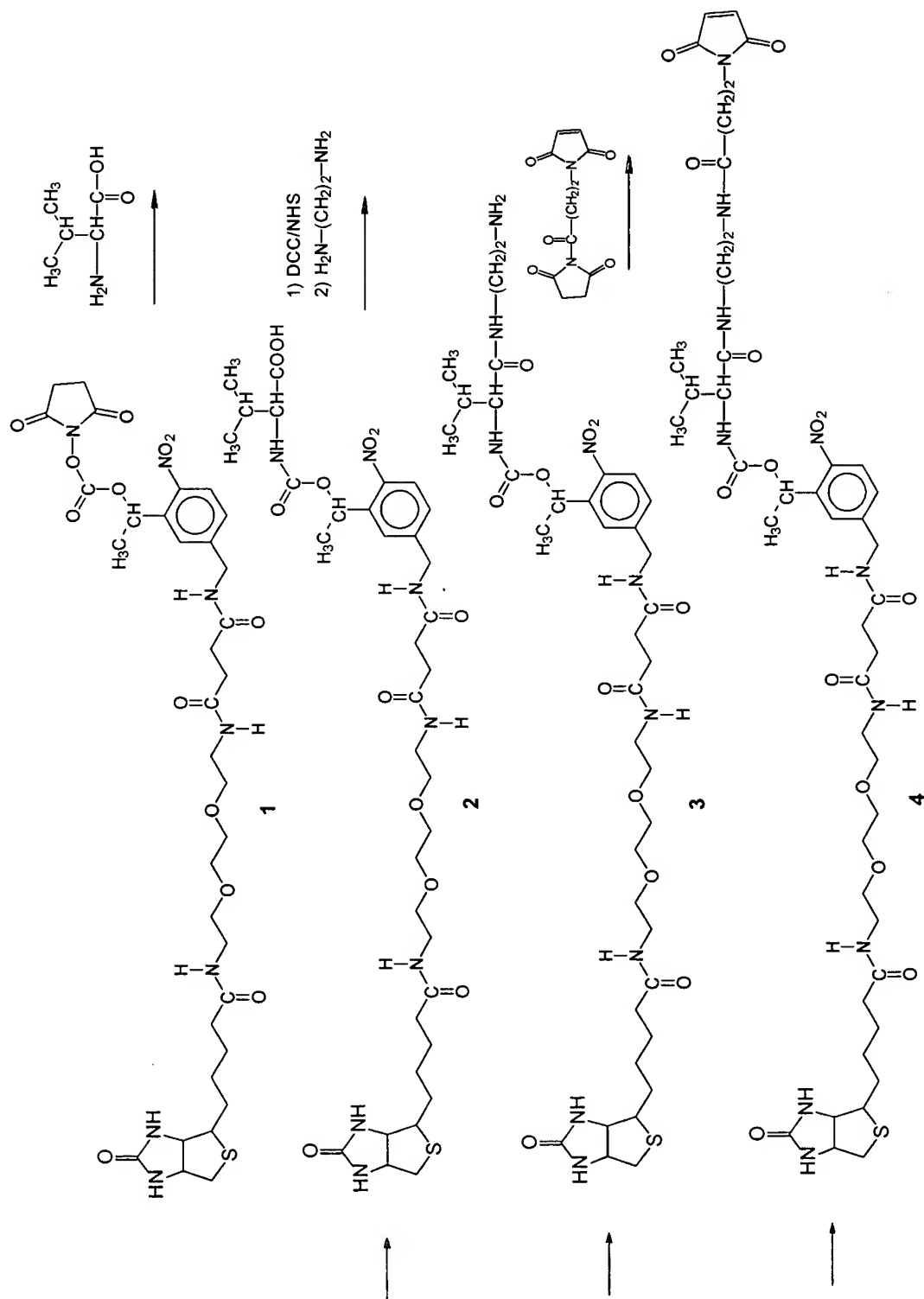


Figure 14C

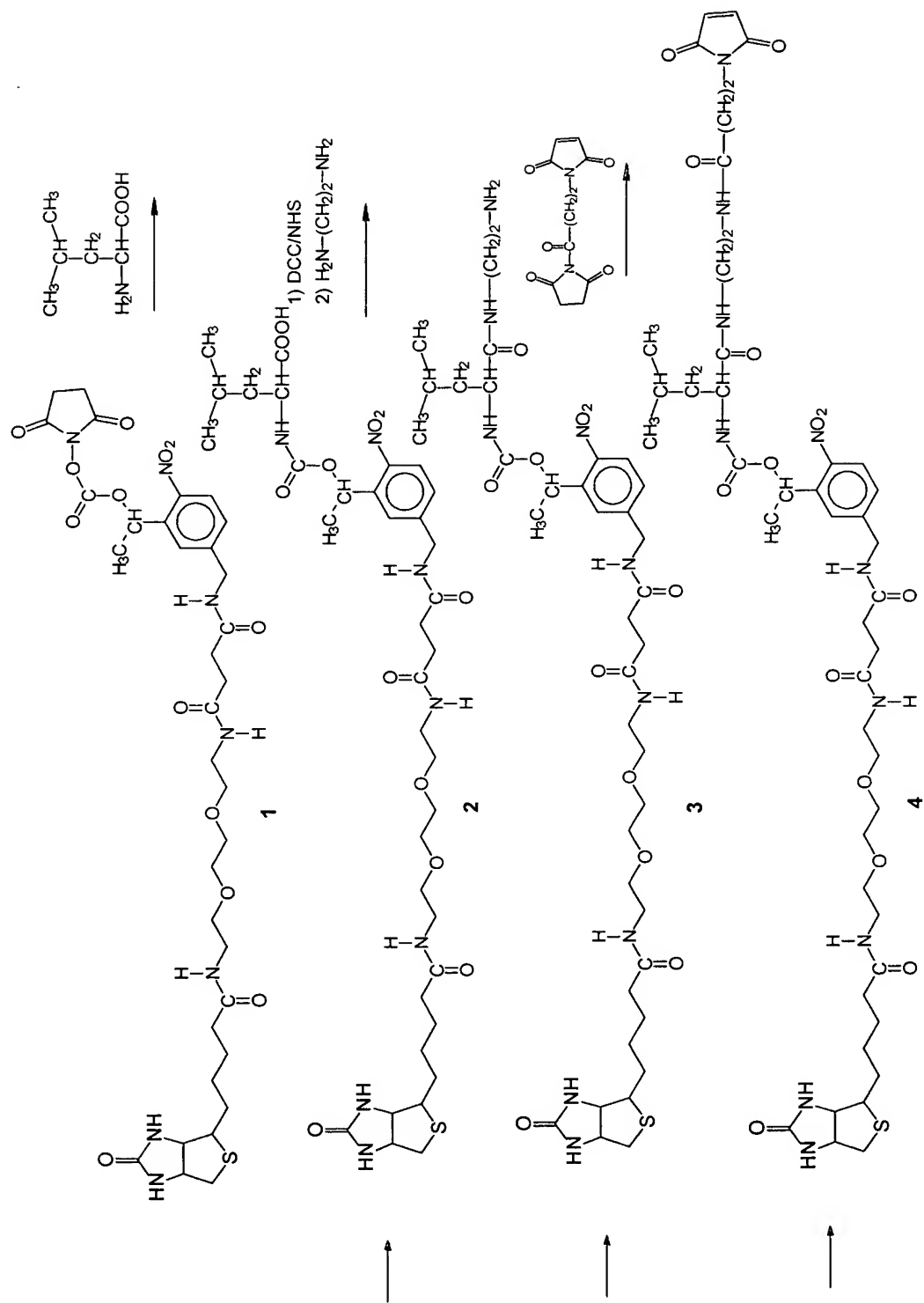
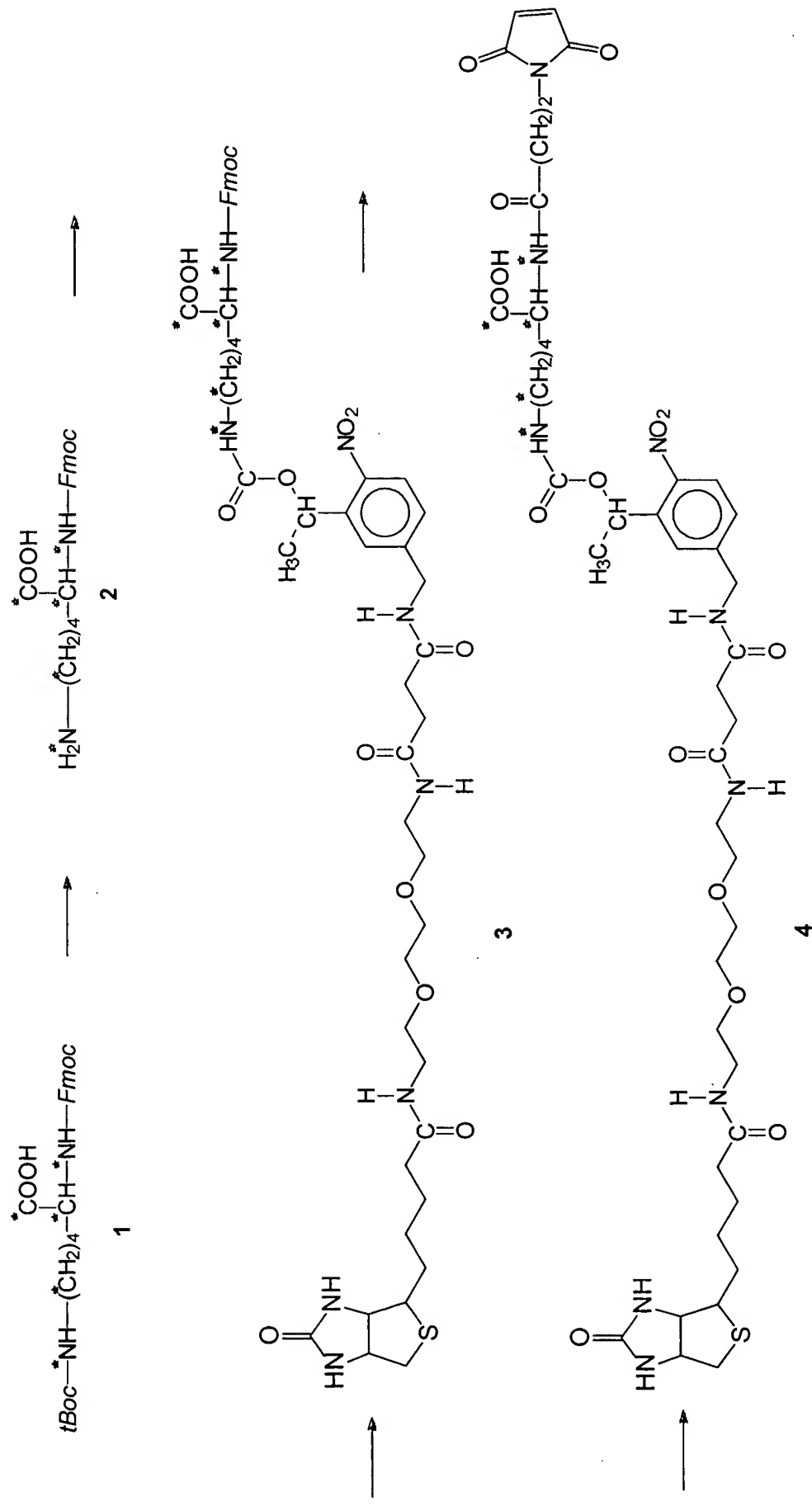




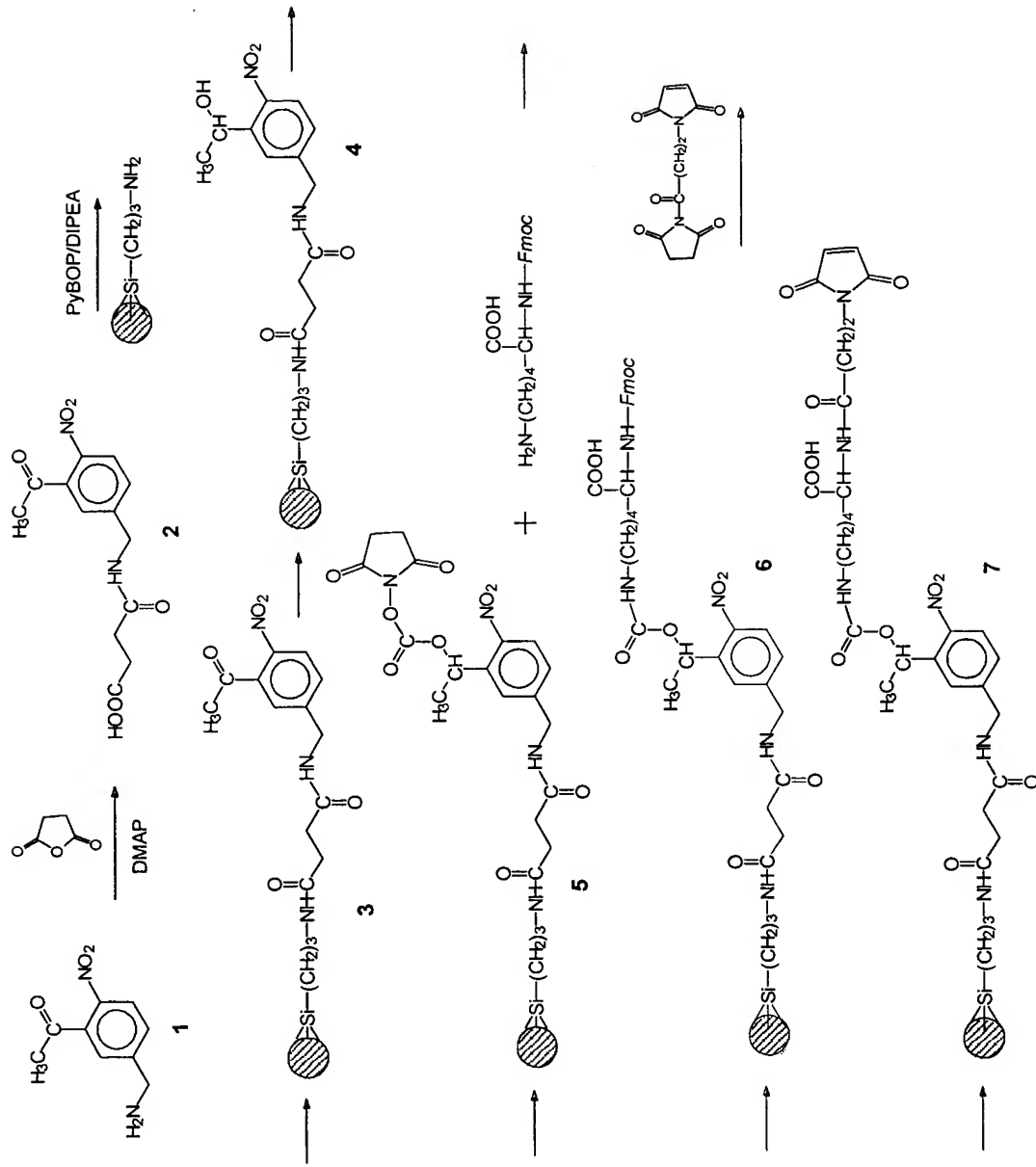
Figure 14D



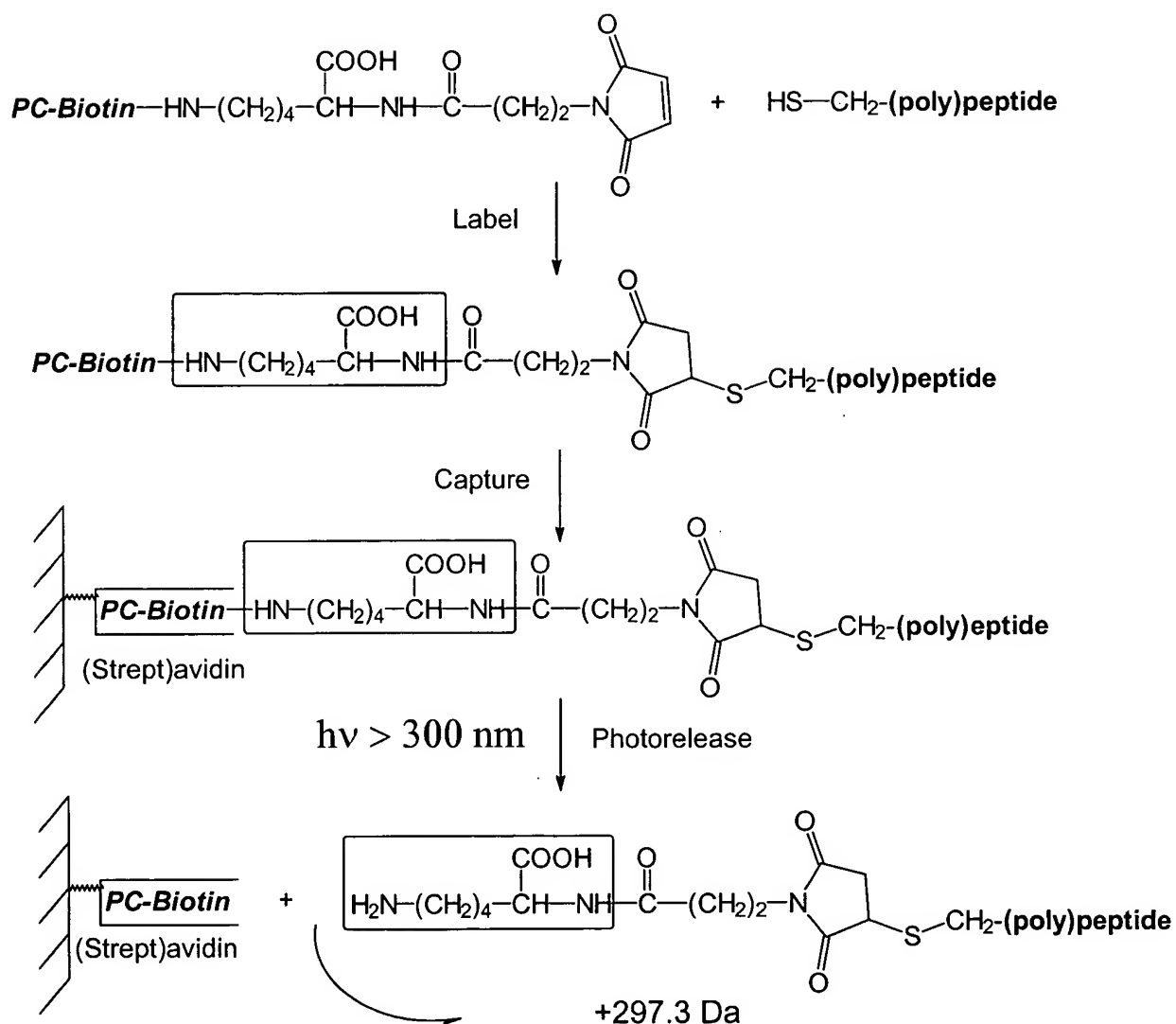
**Figure 14E**

PC-ICAT Reagent	Reactive Group (Reactivity)	Mass change (after cleavage)
<div data-bbox="516 1644 565 1850">PC-ICAT #1</div> <div data-bbox="526 989 724 1770"> <math display="block">\text{PC-Biotin} \text{---} \text{HN} \text{---} \boxed{(\text{CH}_2)_4 \text{---} \text{CH} \text{---} \text{NH} \text{---} \text{C}(=\text{O}) \text{---} (\text{CH}_2)_2 \text{---} \text{N} \text{---} \text{Maleimide}}</math> </div>	Maleimide (Sulphydryls)	297.3
<div data-bbox="800 1644 849 1850">PC-ICAT #2</div> <div data-bbox="812 1125 932 1770"> <math display="block">\text{PC-Biotin} \text{---} \text{HN} \text{---} \boxed{(\text{CH}_2)_4 \text{---} \text{CH} \text{---} \text{NH} \text{---} \text{C}(=\text{O}) \text{---} \text{CH}_2 \text{I}}</math> </div>	Iodacetamide (Sulphydryls)	186.2
<div data-bbox="1027 1644 1076 1850">PC-ICAT #3</div> <div data-bbox="1018 905 1229 1770"> <math display="block">\text{PC-Biotin} \text{---} \text{HN} \text{---} \boxed{(\text{CH}_2)_4 \text{---} \text{CH} \text{---} \text{NH} \text{---} \text{C}(=\text{O}) \text{---} (\text{CH}_2)_3 \text{---} \text{O} \text{---} \text{NHS ester}}</math> </div>	NHS ester (Amines)	242.3

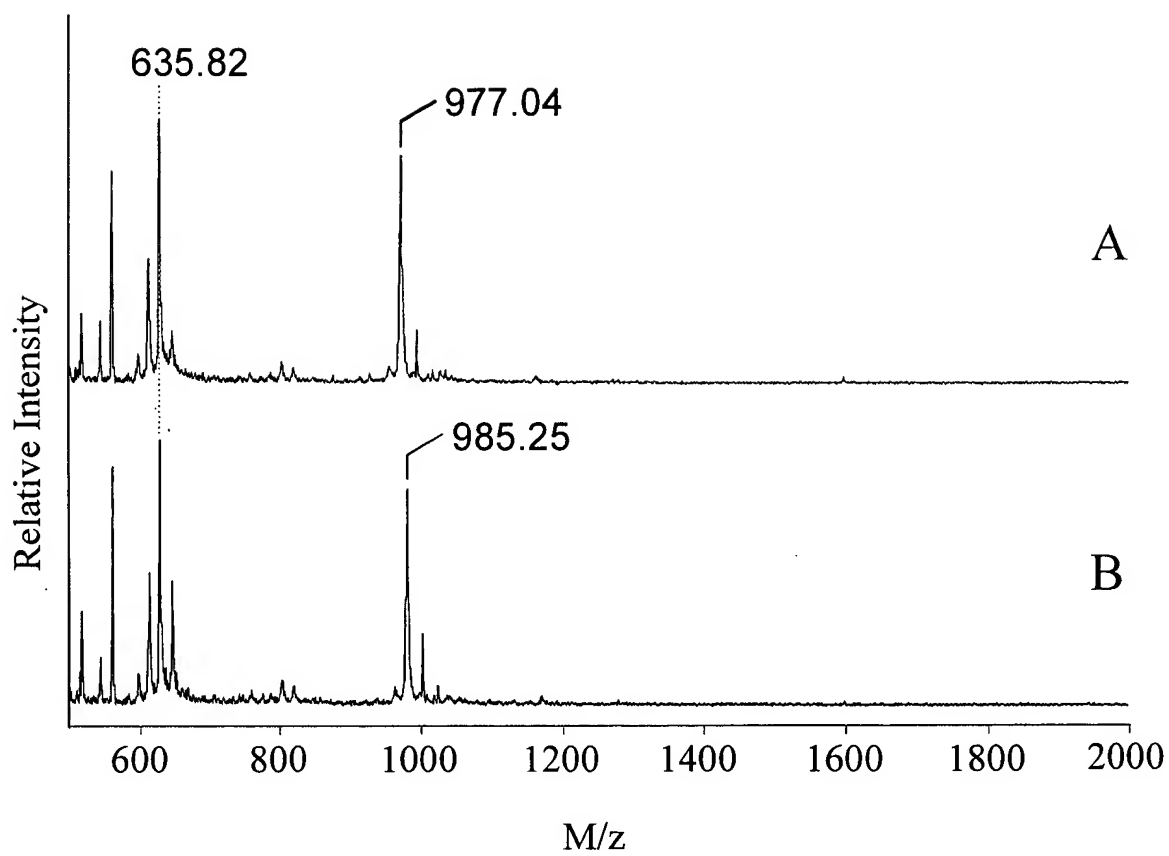
Figure 14F



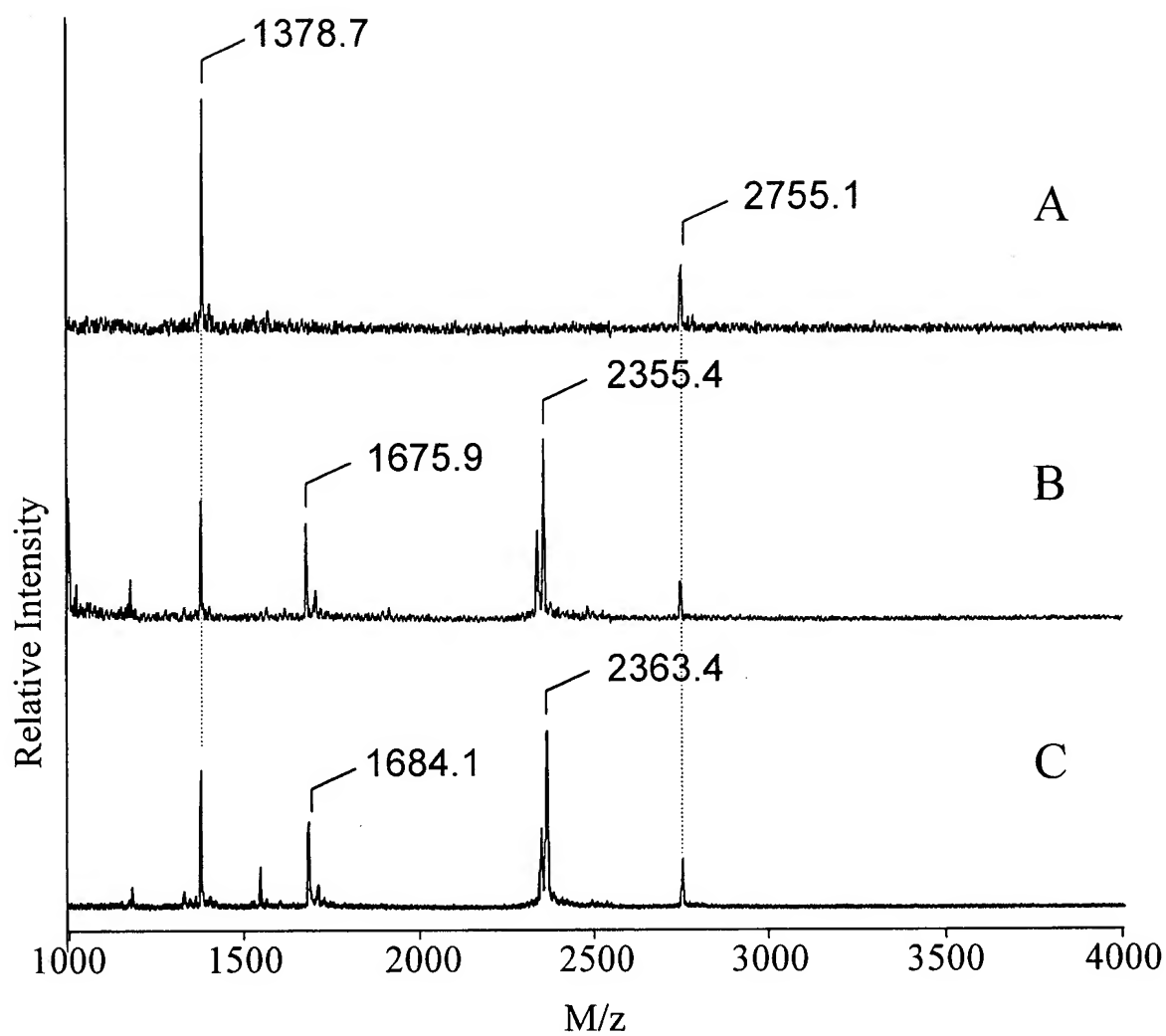
**Figure 15**



**Figure 16**



**Figure 17**



**Figure 18**

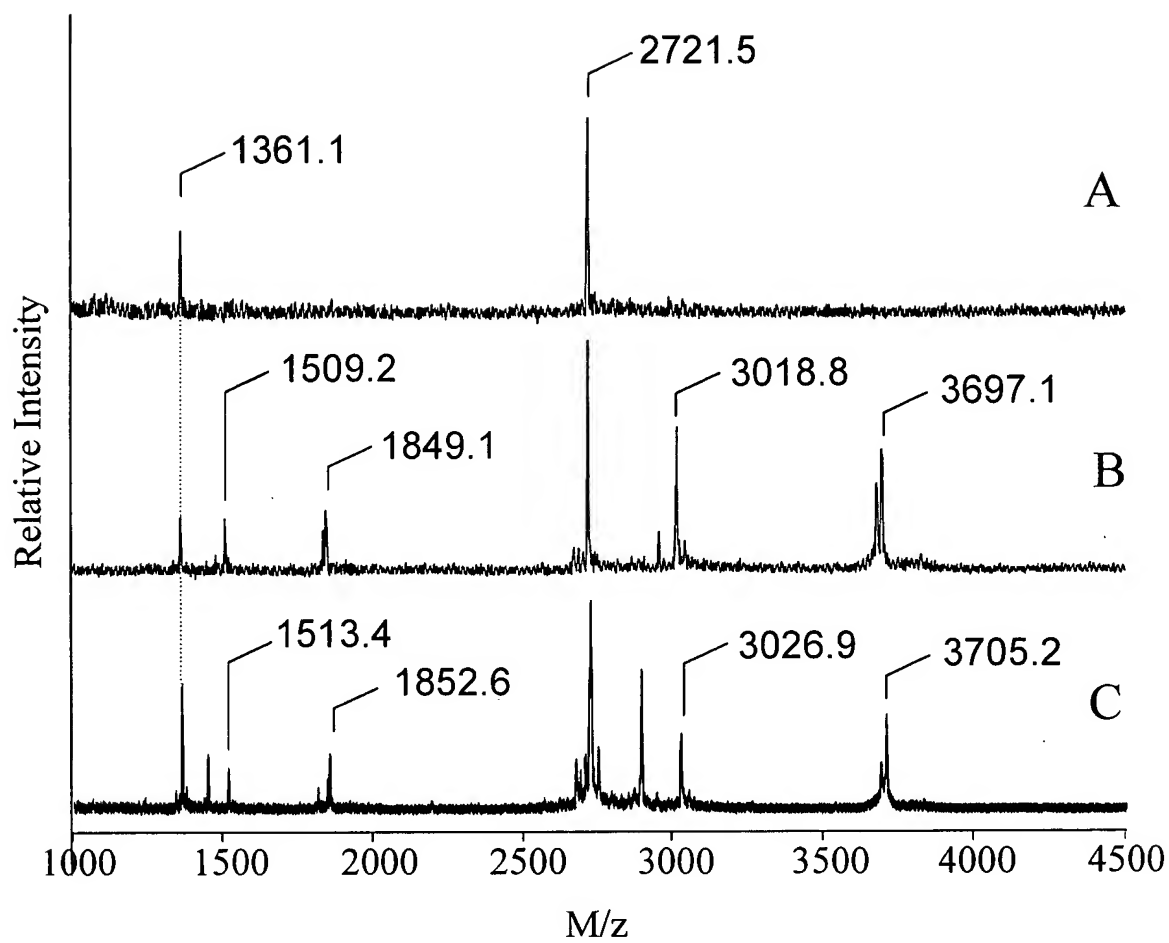
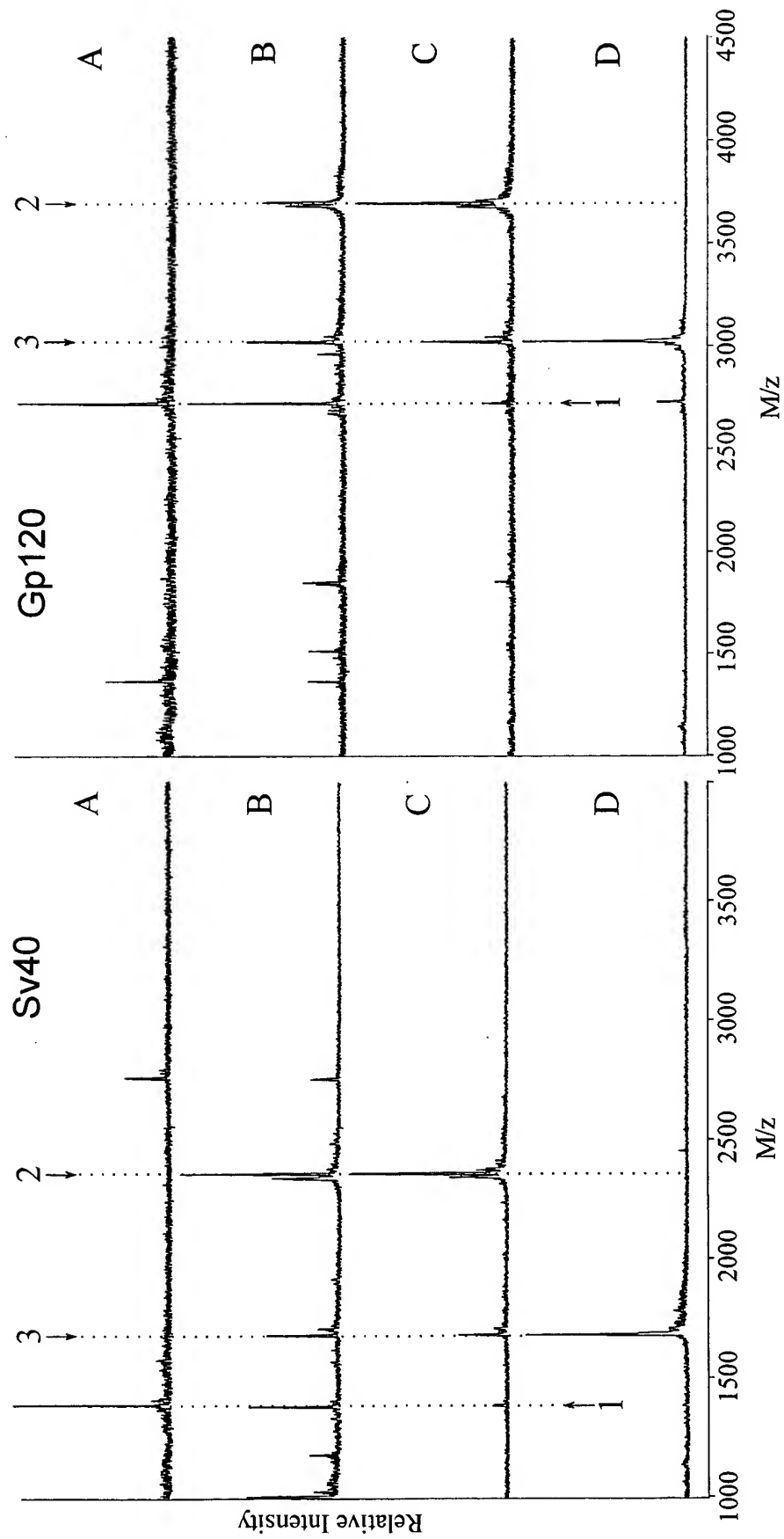


Figure 19

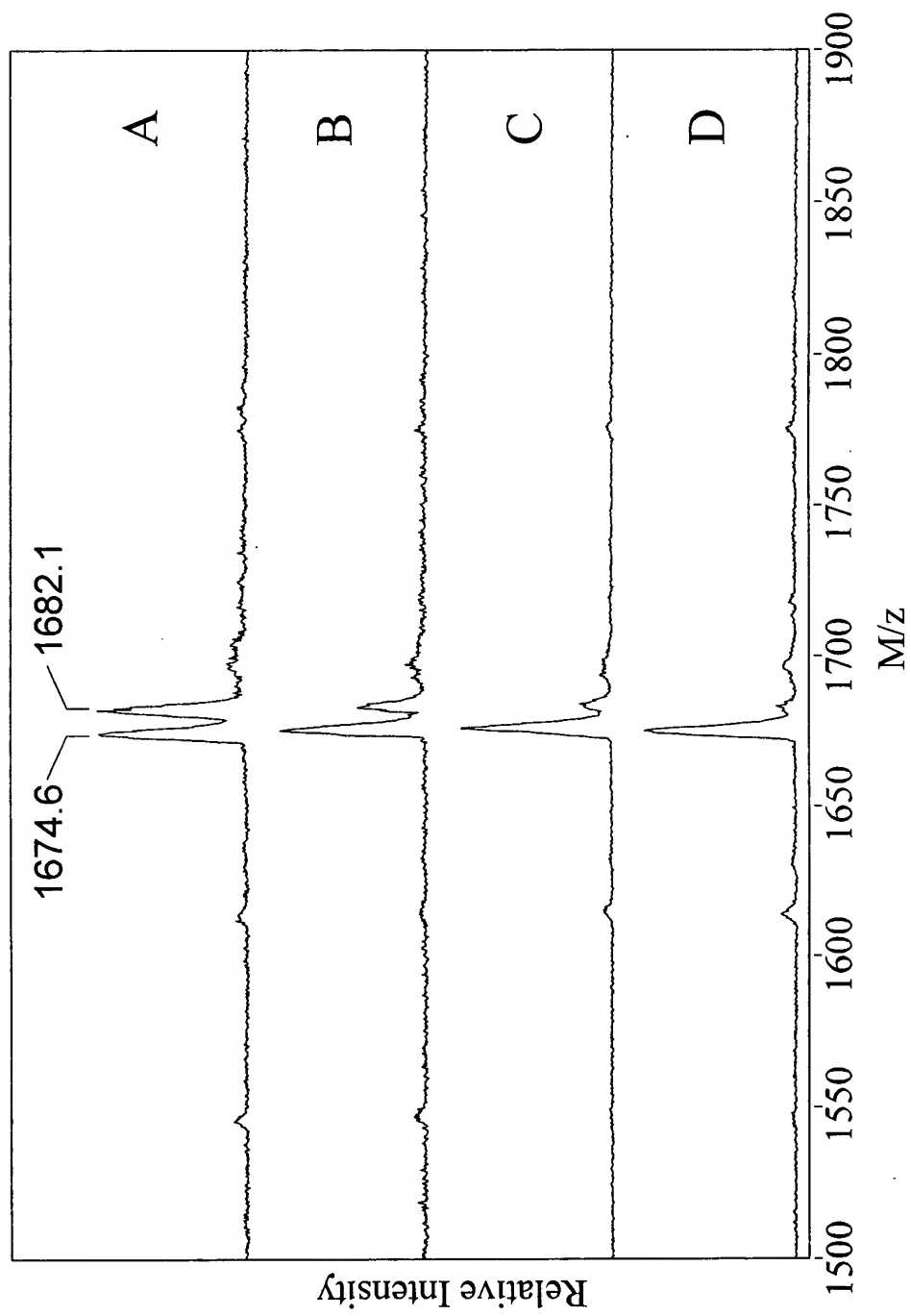




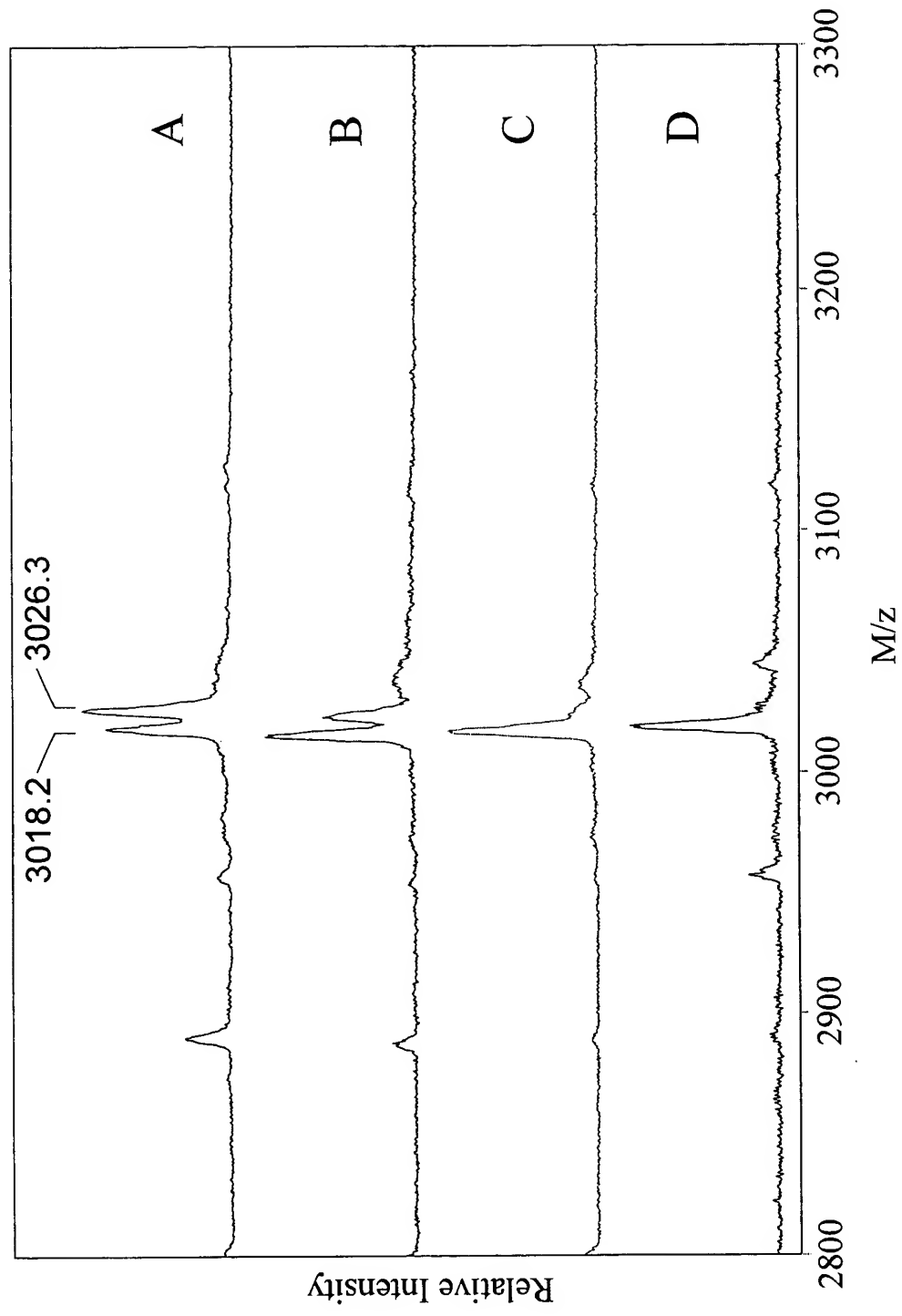
**Figure 20**

Analyte	Mass calculated (MH) <sup>+</sup>	Mass measured (solution)	Mass measured (Streptavidin beads)
Reagent PC-ICAT#1	976.41 (uncleaved); 635.28 (cleaved)	976.94; 635.66	N/a
SV-40 peptide analog	1378.69; 2755.38 (homodimer)	2755.06 (homodimer); 1378.74;	N/a
SV-40 peptide analog+ PC-ICAT#1	2355.1 (uncleaved); 1676.0 (cleaved)	2755.53 (homodimer) 2355.36; 1675.98; 1378.36	Streptavidin beads: 2355.45; 1676.33; Photocleaved/supernatant: 1676.23
HIV gp120 peptide	2721.30	2721.52; 1361.14 (MH <sub>2</sub> ) <sup>+2</sup>	N/a
HIV gp120 peptide + PC-ICAT#1	3697.71 (uncleaved) 3018.61 (cleaved)	3697.09; 3018.82; 2721.45; 1849.13 (uncleaved (MH <sub>2</sub> ) <sup>2+</sup> ); 1509.18 (cleaved, MH <sub>2</sub> ) <sup>2+</sup> ); 1360.88 (MH <sub>2</sub> ) <sup>+2</sup>	Streptavidin beads: 3697.13; 3018.62; Cleaved/supernatant: 3018.89

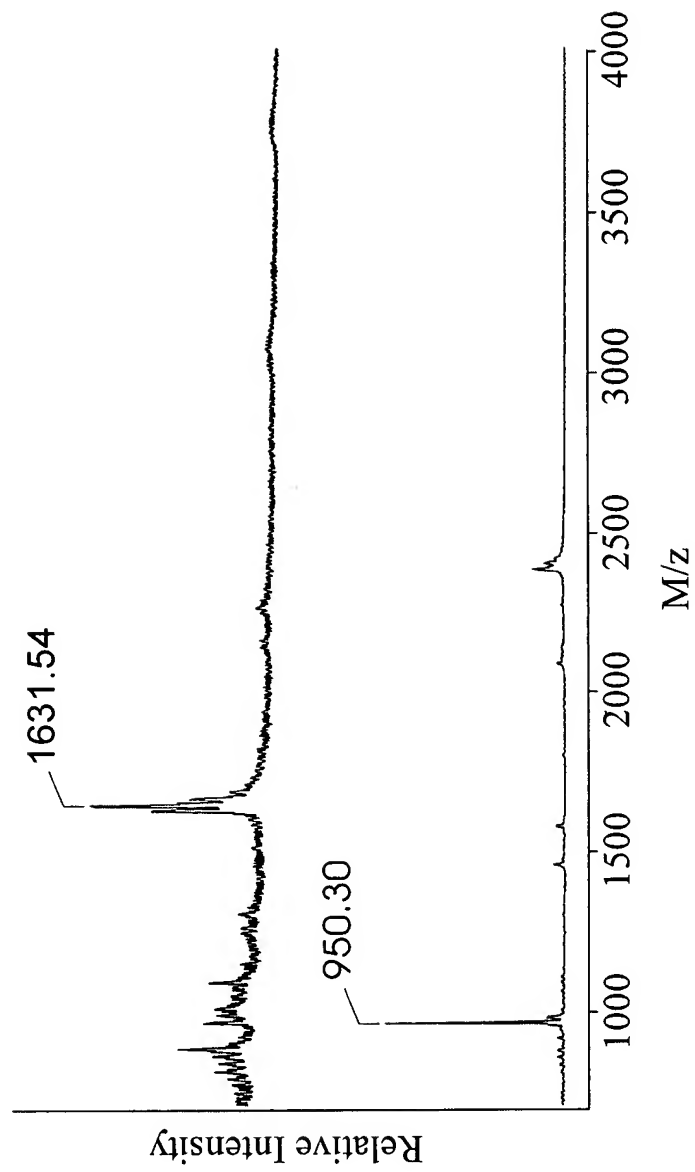
**Figure 21**



**Figure 22**



**Figure 23A**



**Figure 23B**

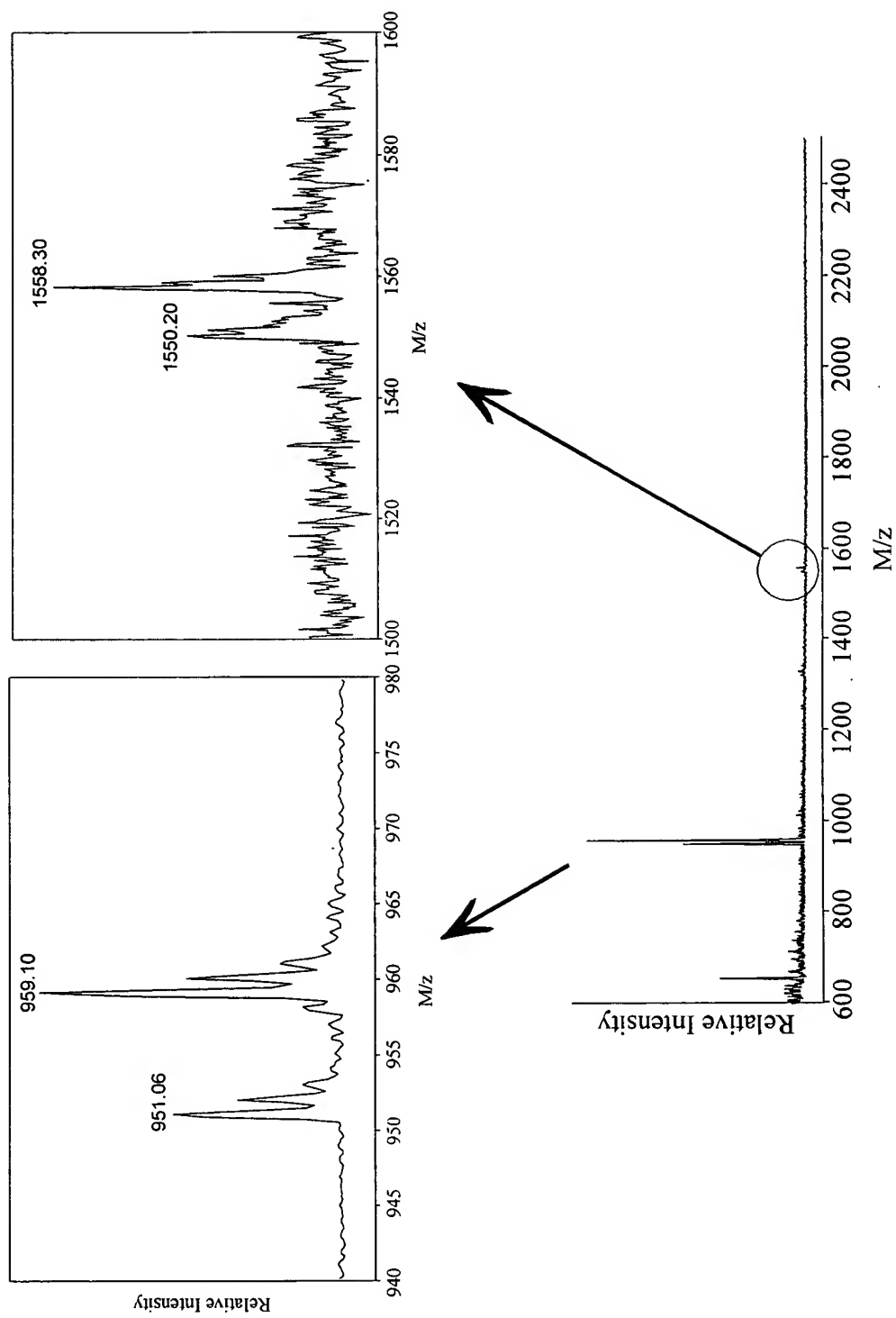
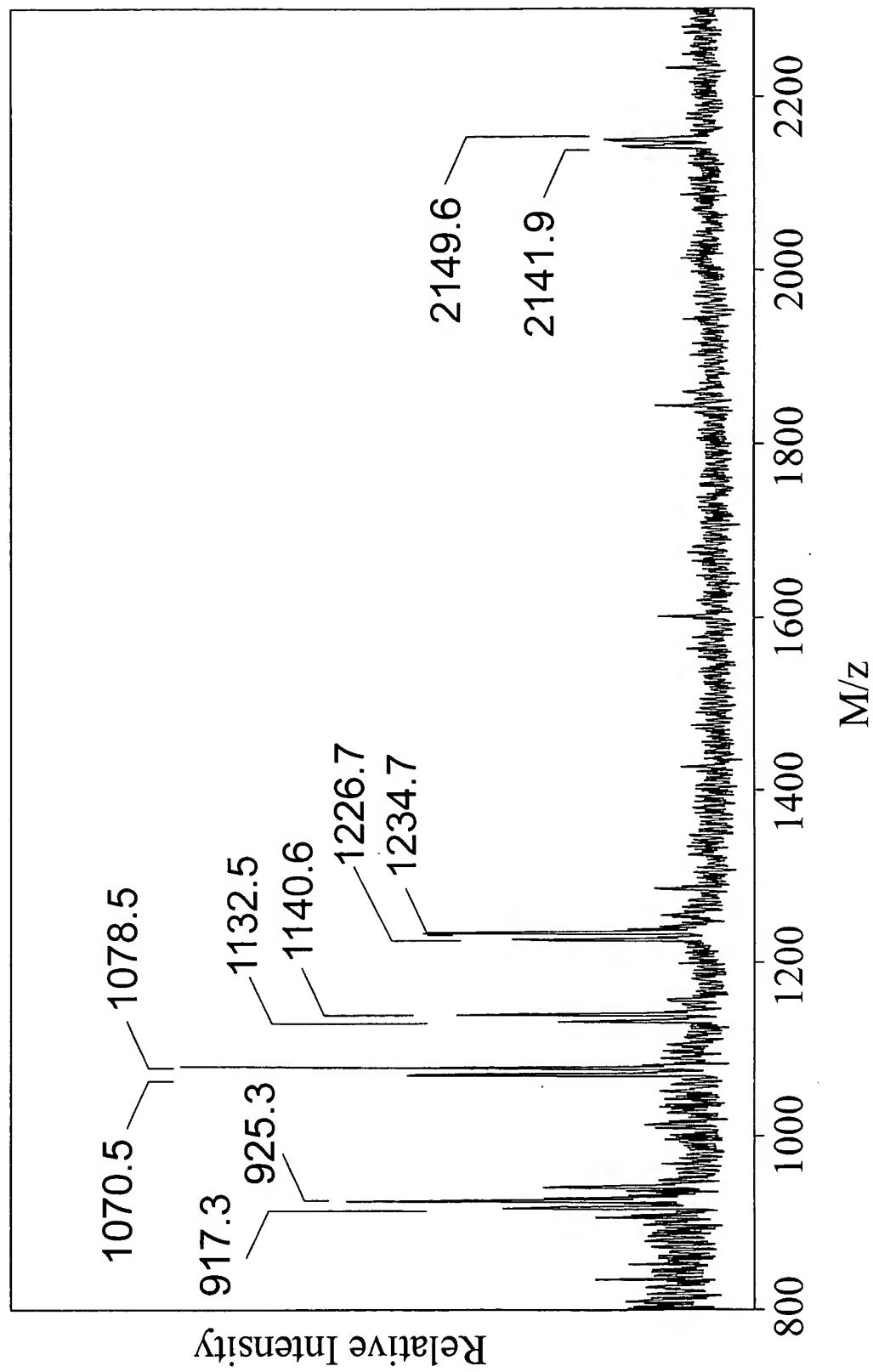
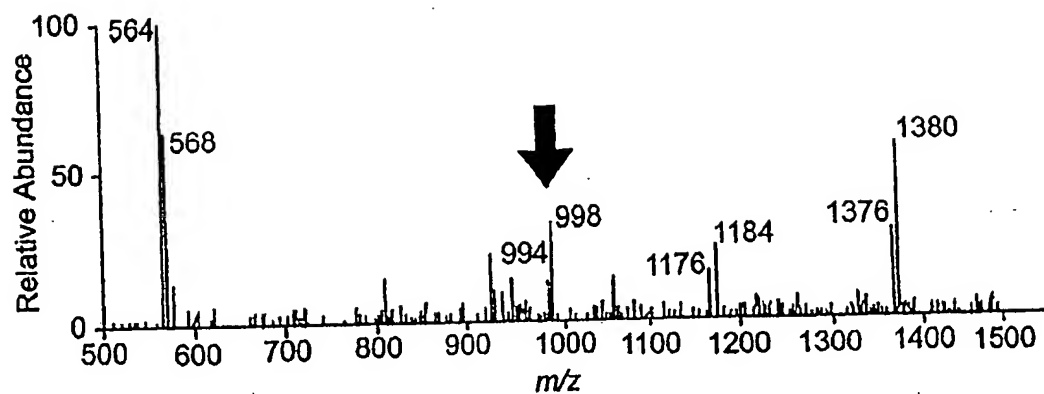


Figure 24

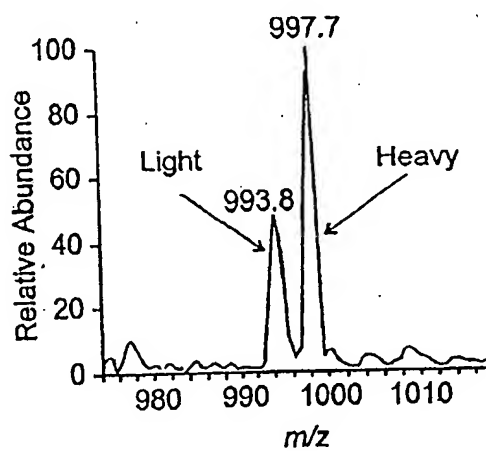


**Figure 25**

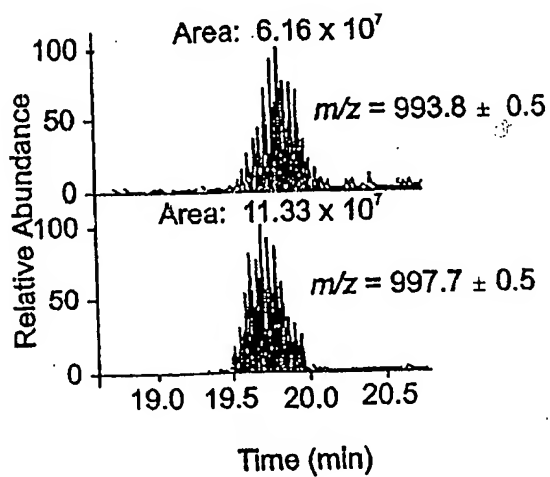
Peptide	H:L ratio (theory)	H:L ratio (experiment)	Standard Deviation	Error, [%]
HIV gp120	1	1.4898	0.126	48.0
	0.50	0.7838	0.038	56.0
	0.20	0.2403	0.004	20.0
	0.10	0.1579	0.043	9.0
SV40	1	1.0593	0.019	6.0
	2	1.9220	0.024	4.0
	5	3.7449	0.362	25.0
	10	5.7204	0.250	56.0



**Figure 26A**



**Figure 26B**



**Figure 26C**



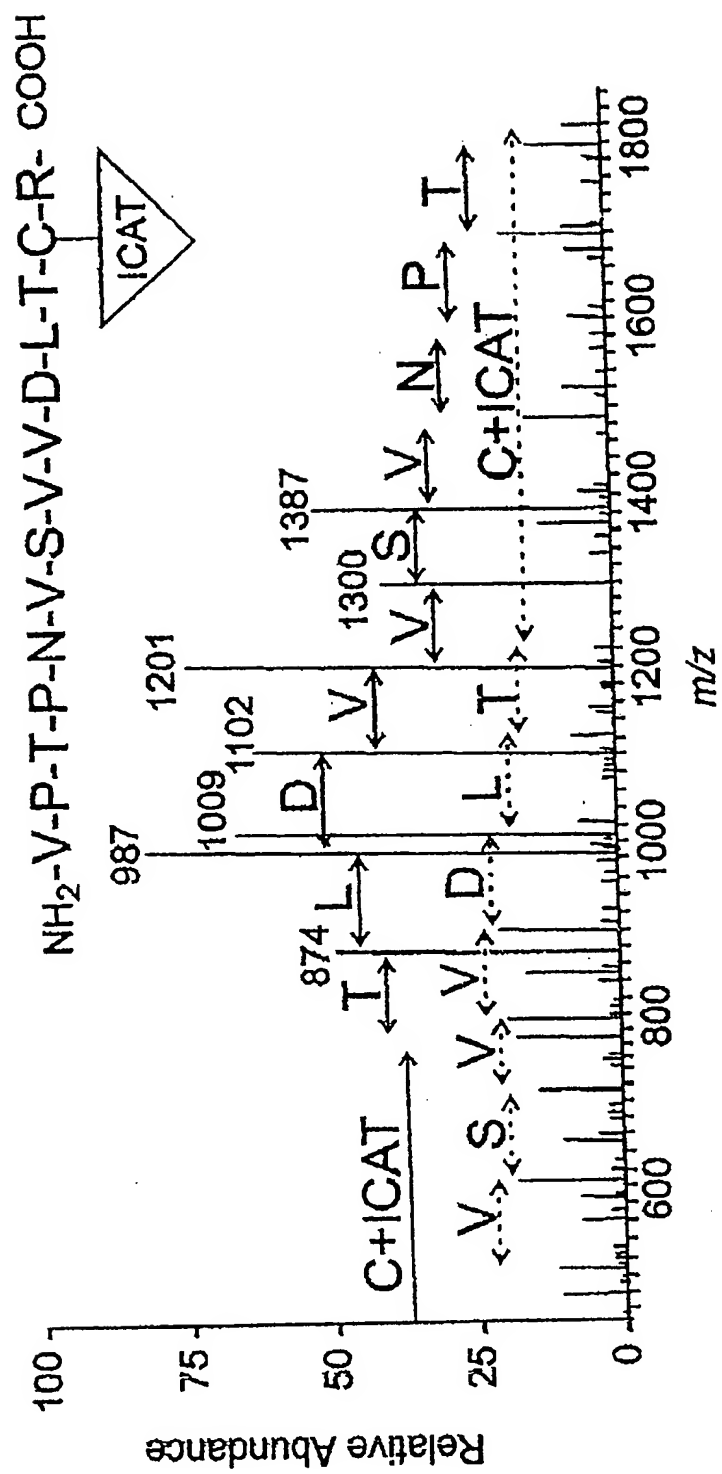
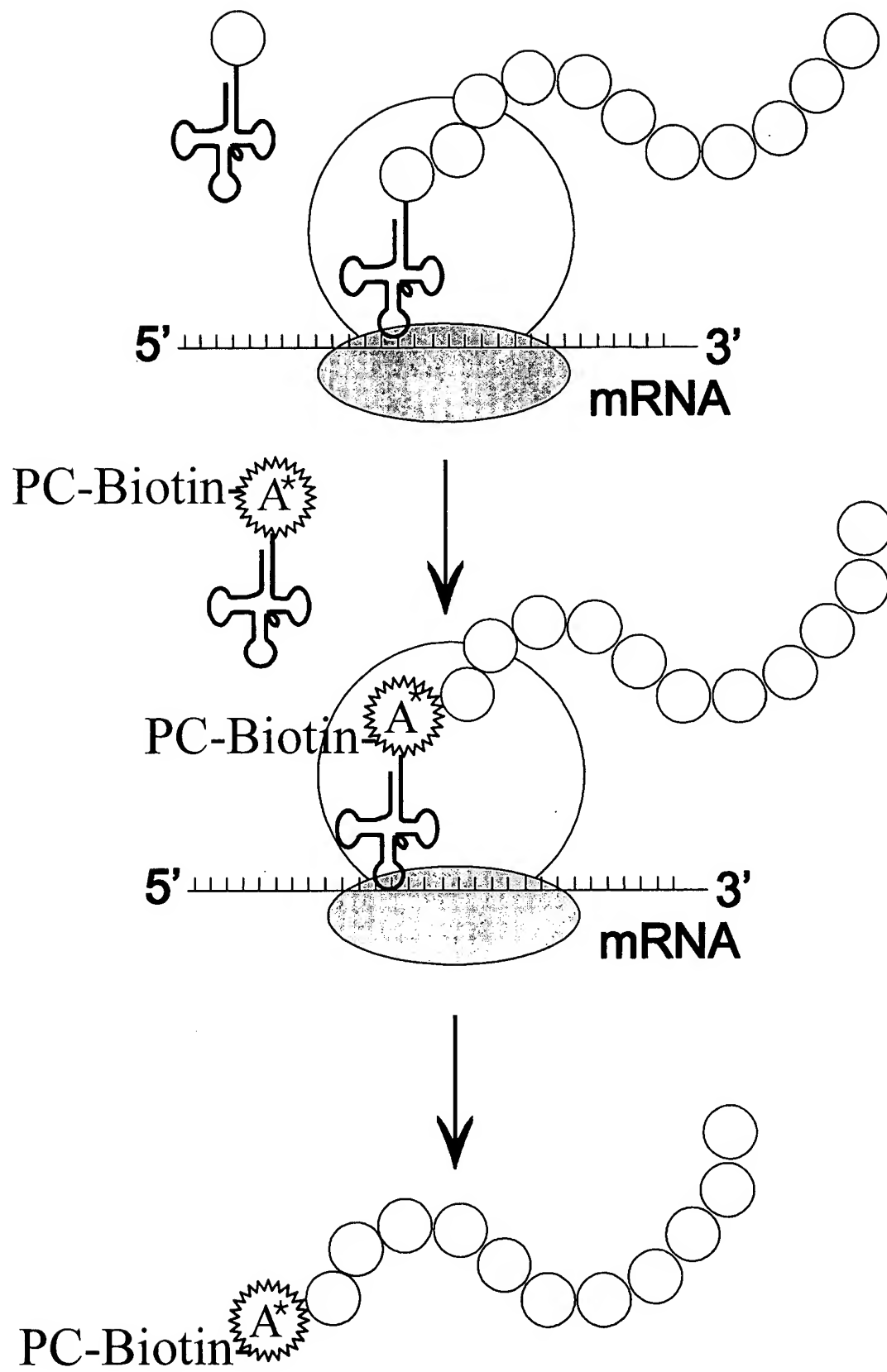


Figure 27A

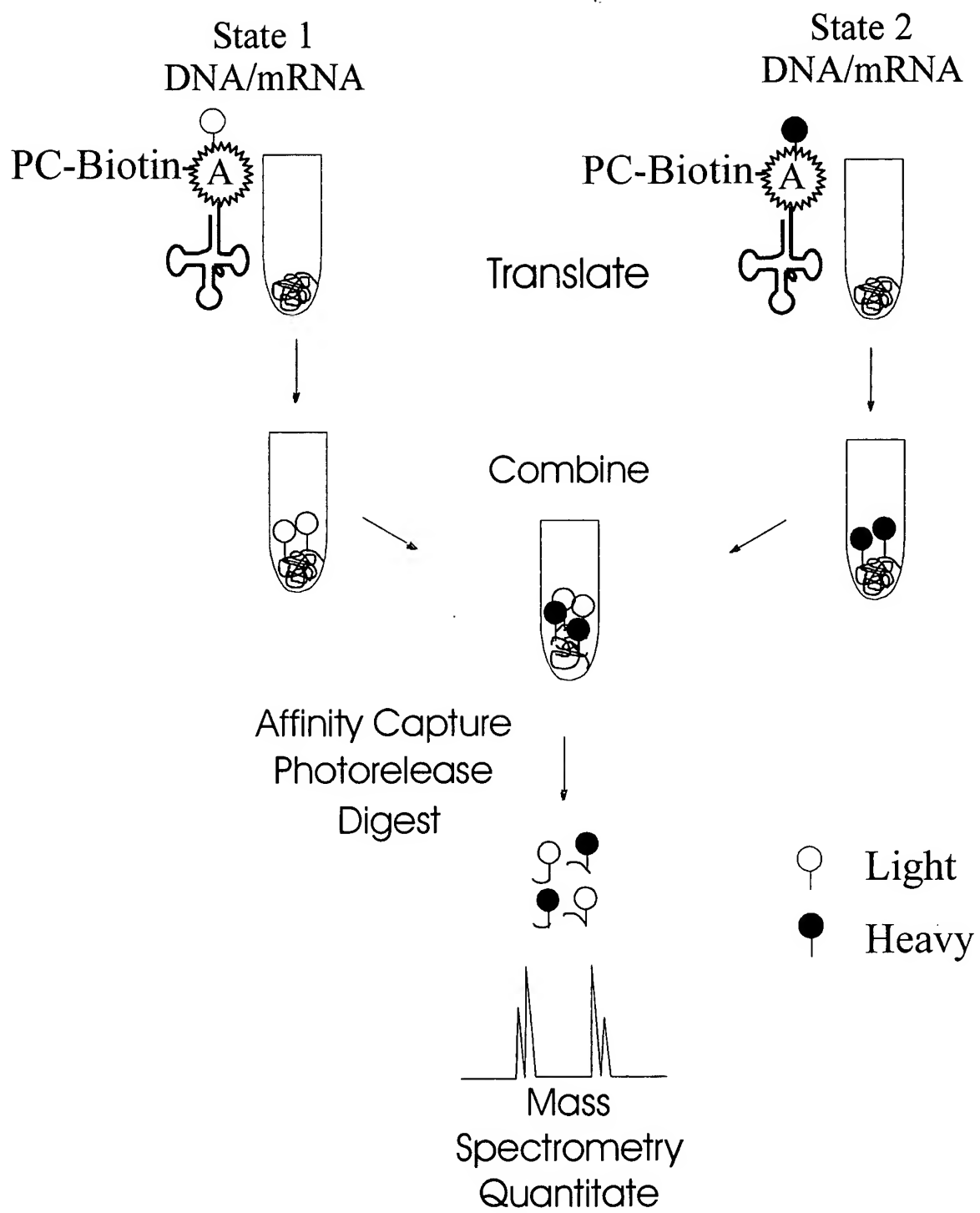
#	<u>Rank/Sp</u>	<u>(M+H)<sup>+</sup>C*10<sup>4</sup></u>	<u>Ions</u>	<u>Reference</u>	<u>Peptide</u>
1.	1/ 1	1994.3 4.4675	17/26	G3P_RABIT	(R)VPTPNVSVVDLTC#R (SEQ ID NO:60)
2.	2/ 403	1995.1 2.7366	13/34	SLTRNGL	(E)LGKPVLTANQVTIWEGLR (SEQ ID NO:61)
3.	3/ 3	1995.0 2.6591	16/36	FLP_LACCA	(N)LANPNVYTETLTAAATVCTI (SEQ ID NO:62)
4.	4/ 209	1995.0 2.6335	14/36	A42912	(Y)LALLPSDAEGPHGQFVTDK (SEQ ID NO:63)
5.	5/ 381	1995.1 2.4634	13/38	H69373	(L)ALLVLVAPAMAAAGNGEDLRN (SEQ ID NO:64)

**Figure 2 7B**

**Figure 28**



**Figure 29**



**Figure 30**

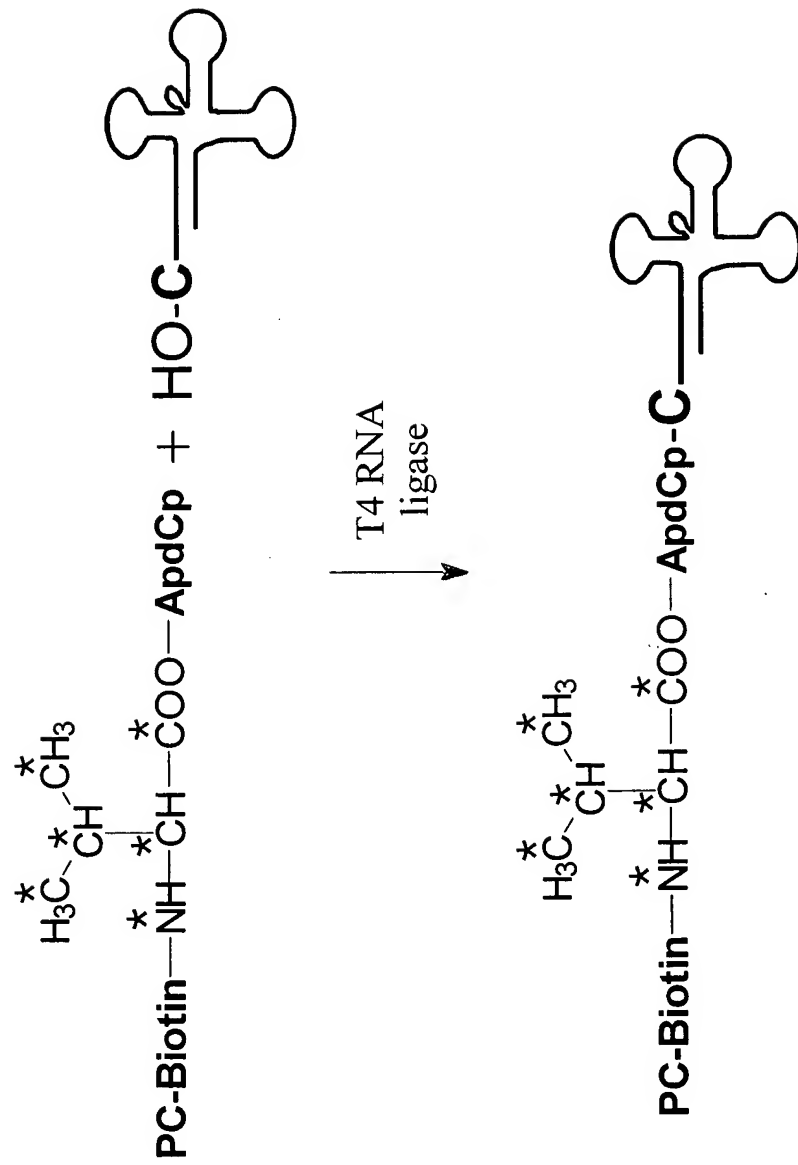




Figure 32

